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INTERCROPPING FABA BEAN WITH SOME LEGUME CROPS FOR CONTROL OF *OROBANCHE CRENATA*

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Experiments involving the intercropping of faba bean with each of lupin, fenugreek and Egyptian clover as well as growing faba bean alone were carried out at Assiut University on a farm naturally infested with *Orobanche* in two seasons. The major objective of the study was to investigate the effect of different intercropping combinations with faba bean on the infestation with *Orobanche*. The results obtained revealed that intercropping faba bean with each of lupin, fenugreek and Egyptian clover markedly reduced the *Orobanche crenata* Forsk. infestation of faba bean. The number of branches, the height of the first pod, the number of pods, the seed yield and the number and dry weight of *Orobanche* spikes were significantly affected by the intercropping treatments, but these had no significant influence on plant height, straw yield or 100-seed weight. Intercropping faba bean with each of lupin, fenugreek and Egyptian clover increased the faba bean seed yield, consequently the economic return was also increased.

Key words: faba bean, intercropping, *Orobanche crenata*, infestation

Introduction

In the Nile valley of Egypt, faba bean fields are often infested with *Orobanche* spp. In Middle and Upper Egypt, the level of infestation is so high that there has been complete crop failure (Telaye and Saxena, 1986). In Sicily, broomrape (*Orobanche crenata* Forsk) is the most serious biotic stress of faba bean, frequently limiting the faba bean yield and sometimes destroying the crop completely (Polignano, 1979).

The germination of *Orobanche* is stimulated by root exudates from faba bean roots, so it is difficult for researchers to tackle the problem (Al-Menoufi, 1989).

Chemical and biological methods to control the parasite were investigated by Nassib et al. (1985) and Linke et al. (1990). Another approach by using trap plants through intercropping as a possible method to control *Orobanche* was reported by Radwan et al. (1988) and Al-Menoufi et al. (1991).

Al-Menoufi (1991) reported that intercropping faba bean (*Vicia faba* L.) with the leguminous forage crop fenugreek (*Trigonella foenum-graecum* L.) markedly reduced *Orobanche* infestation. Also, he reported that Egyptian clover had been proposed as a trap crop for *Orobanche*. In contrast, however, Khalaf (1994) concluded that intercropping faba bean with fenugreek did not cause any decrease in *Orobanche* infestation, though he reported that Egyptian clover, which is only rarely infested by *Orobanche*, may be used as a trap crop.

The objective of this work was to investigate the effect of intercropping faba bean with legume crops on the infestation of *Orobanche*. The yield of faba bean and the response of the crop to intercropping were considered with the aim of increasing the net income of farmers.

Materials and methods

The present experiments were conducted to find a suitable crop for intercropping with faba bean and a suitable intercropping system for tolerance to *Orobanche*.

The experiments were carried out in the Experimental Station of Assiut University, where the soil was naturally infested by *Orobanche*. The soil of the experimental site was clay in texture with an average pH of 7.8, saturation capacity 42%, organic matter 1.80%, available phosphorus (ppm) 9.0 and total nitrogen 0.08%.

Seeds of faba bean cv. Giza 429 (main crop) were planted on October 20 and 21 in 1998 and 1999, respectively, at interrow spacing of 60 cm and interplant spacing of 10 cm with two plants/hill. The intercrops (lupin, fenugreek or Egyptian clover) were drilled on the other side of the ridge using the recommended seeding rate at the same time as the main crop in a randomized complete block design with four replications.

The experimental treatments were:

1. Faba bean alone (control).
2. Faba bean + lupin (*Lupinus termis*) cv. Giza 2.
3. Faba bean + fenugreek (*Trigonella foenum-graecum*) cv. Giza 2.
4. Faba bean + Egyptian clover (*Trifolium alexandrinum* L.) cv. Fahl.

At harvest, the following characters were recorded on a random sample of 20 guarded faba bean plants in each plot:

- | | |
|-----------------------------|---------------------------------|
| 1. Plant height, cm | 2. Height of the first pods, cm |
| 3. Number of branches/plant | 4. Number of pods/plant |
| 5. Seed yield/plant, g | |

In addition the following characters were recorded on all plants in each plot for the main crop:

- | | |
|--|---|
| 6. Seed yield, ton/hectare | 7. Straw yield, ton/hectare |
| 8. 100-seed weight, g | 9. Number of <i>Orobanche</i> spikes/m ² |
| 10. Dry weight of <i>Orobanche</i> spikes/m ² . | |

The seed and straw yields of the intercrops were also recorded.

The results were statistically analysed according to Gomez and Gomez (1984) for each single season and combined analysis was also done. The comparisons of means were carried out using L.S.D. at the 5% probability level.

Results and discussion

The Bartlett test for the homogeneity of the error indicated that the variance of the data of both seasons was insignificant. Thus, the combined analysis was carried out.

The combined analysis of variance for the studied traits is presented in Table 1. Highly significant differences were obtained between the growing seasons for plant height, height of the first pod, number of branches/plant, seed and straw yields and seed index.

Table 1

Mean squares from combined analysis of variance for the studied traits of four intercropping systems for the two seasons

Traits	Source of variations				
	Years (Y)	Rep./years	Intercropping (I)	I × Y	Error
D.F.	1	6	3	3	18
Plant height, cm	4074.1**	43.92	27.10	20.35	19.93
Height of first pod, cm	979.7**	9.65	130.7**	6.35	13.63
No. of branches/plant	1.20**	0.04	0.47**	0.06	0.07
No. of pods/plant	0.18	3.86	14.98**	6.53*	2.03
Seed yield/plant, g	18.60**	0.31	7.92**	3.56**	0.60
Seed yield, t/ha	5.63**	0.12	6.02**	0.87	0.43
Straw yield, t/ha	21.98**	1.42	0.34	1.13	0.74
100-seed weight, g	82.6**	3.46	16.3	4.23	7.12
No. of <i>Orobanche</i> spikes/m ²	81.3	45.3	222.7**	41.99**	6.89
Dry weight of <i>O.</i> spikes/m ² , g	19.52	60.6	1353.5**	214.68**	39.42

*, and **: significant at the 5% and 1% levels of probability, respectively.

The results in Table 2 indicated that the intercropping system exhibited a highly significant effect on the number and dry weight of *Orobanche* spikes/m². This means that intercropping treatments would help to reduce the infestation of *Orobanche* in the faba bean field. The number of branches, height of the first pod, number of pods, seed yield and number and dry weight of *Orobanche* spikes/m² were significantly affected by intercropping faba bean with each of lupin, fenugreek and Egyptian clover, while plant height, straw yield and 100-seed weight were not significantly affected by the intercropping systems studied.

These effects of intercropping faba bean with lupin, fenugreek and Egyptian clover increased the seed yield. This may be due to the increase in the number of pods/plant, seed yield/plant and seed index and the reduction in the number and dry weight of *Orobanche* spikes/m².

The decrease in *Orobanche* infestation by planting lupin or fenugreek may be due to the fact that these plants secrete some chemicals which inhibit the germination of *Orobanche* seeds or prevent the infestation of faba bean by *Orobanche*. The available results are in agreement with those of Al-Menoufi (1991) who reported that intercropping fenugreek with faba bean markedly reduced the *Orobanche* infestation of faba bean. Also, he reported that fenugreek and Egyptian clover produced germination stimulants and could be used as a trap crop. These results are not in agreement with those of Khalaf (1994), who reported that intercropping faba bean with fenugreek did not cause a decrease in *Orobanche* infestation. It can be hypothesised that this researcher worked with a different strain of *Orobanche crenata*, which, in contrast to the strain used in the present study, was able to attack fenugreek. Differences between fenugreek varieties may also have played a role in this respect.

The determination of the economic return for each intercropping system and for faba bean planted alone is recorded in Table 3. The data revealed that the economic return increased when intercropping faba bean with lupin, fenugreek or Egyptian clover. It is preferable to intercrop lupin or fenugreek with faba bean in soil infested with *Orobanche*.

Table 2

Effect of intercropping systems on the yield and yield components of faba bean and on *Orobanche* infestation

Yield components	Intercropping system						F-test	LSD _{5%}
	FB	FB + L	FB + F	FB + E	Mean			
Plant height (cm)								
1998/1999	142.8	147.3	150.8	147.3	147.0	NS	—	
1999/2000	124.8	123.2	125.7	124.0	124.4	NS	—	
Combined	133.8	135.2	138.2	135.6		NS	—	
Height to first pod (cm)								
1998/1999	52.8	44.2	46.2	46.1	47.3	*	5.33	
1999/2000	64.8	56.50	57.80	54.50	58.40	*	6.43	
Combined	58.80	50.30	52.00	50.30		**	3.88	
No. of branches/plant								
1998/1999	2.48	2.18	2.25	2.05	2.24	*	0.28	
1999/2000	2.33	1.68	1.73	1.68	1.85	N.S.	—	
Combined	2.40	1.93	1.99	1.86		*	0.28	
No. of pods/plant								
1998/1999	9.11	12.63	10.73	8.07	10.13	**	1.96	
1999/2000	8.30	11.00	10.10	10.55	9.99	NS	—	
Combined	8.70	11.82	10.42	9.31		**	1.49	
Seed yield/plant								
1998/1999	12.10	17.82	16.64	16.98	15.81	**	3.72	
1999/2000	10.86	16.38	14.85	12.05	13.54	**	1.50	
Combined	11.48	16.95	15.74	14.52		**	1.86	
Seed yield, t/ha								
1998/1999	2.83	3.73	3.96	2.92	3.36	**	0.50	
1999/2000	2.42	2.86	2.93	2.98	2.80	*	0.41	
Combined	2.63	3.29	3.44	2.95		**	0.30	
Straw yield, t/ha								
1998/1999	4.76	4.95	5.47	5.24	5.10	NS	—	
1999/2000	4.19	3.09	3.19	3.33	3.45	NS	—	
Combined	4.48	4.02	4.33	4.28		NS	—	
100-seed weight, g								
1998/1999	63.40	66.95	67.33	67.38	66.26	NS	—	
1999/2000	61.76	64.51	62.44	63.49	63.05	NS	—	
Comb.	62.58	65.75	64.88	65.43		NS	—	
No. of <i>Orobanche</i> spikes/m ²								
1998/1999	12.40	5.60	6.80	6.00	7.70	**	1.99	
1999/2000	21.8	9.30	7.50	4.95	10.89	**	4.60	
Combined	17.10	7.45	7.15	5.48		**	2.76	
Dry weight of <i>O.</i> spikes/m ² , g								
1998/1999	31.0	14.45	17.0	14.50	19.24	**	4.81	
1999/2000	48.0	11.00	11.90	12.30	20.80	**	10.36	
Combined	39.50	12.72	14.45	13.40		**	5.60	

* and ** significant at 5% and 1% levels of probability, respectively.; NS=non-significant; FB: Faba bean, FB + L: Faba bean + lupin, FB + F: Faba bean + fenugreek; FB + E: Faba bean + Egyptian clover

Table 3

Returns from intercropping systems involving faba bean with each of lupin, fenugreek and Egyptian clover under natural soil infestation with *Orobanche*

	Intercropping system			
	FB	FB + L	FB + F	FB + E
Seed yield of faba bean (t/ha)				
1998/1999	2.83	3.73	3.96	2.92
1999/2000	2.42	2.86	2.93	2.98
Comb.	2.63	3.29	3.44	2.95
Straw yield of faba bean (t/ha)				
1998/1999	4.76	4.95	5.47	5.24
1999/2000	4.19	3.09	3.19	3.33
Comb.	4.48	4.02	4.33	4.28
Seed yield of intercrop (t/ha)				
1998/1999	—	0.96	1.00	0.26
1999/2000	—	0.59	0.50	0.18
Comb.	—	0.77	0.75	0.22
Straw yield of intercrop (t/ha)				
1998/1999	—	2.67	2.26	2.62
1999/2000	—	2.05	2.02	2.19
Comb.	—	2.36	2.14	2.40
Revenue (\$/ha)*				
1998/1999	1139.82	1999.81	2012.66	1457.17
1999/2000	979.00	1443.57	1380.33	1353.60
Comb.	1059.37	1721.69	1696.53	1404.88

*The official prices for these crops according to the Ministry of Agriculture, Cairo, Egypt, 1999; FB: Faba bean, FB + L: Faba bean + lupin, FB + F: Faba bean + fenugreek; FB + E: Faba bean + Egyptian clover

The chemicals secreted by legume roots may stimulate the faba bean plants, which is clear in the increase in plant height, pods/plant, seed yield/plant and per hectare. Also, these substances decrease branching, resulting in an increase in the plant height of the faba bean plants. The most important crops in increasing the yield of faba bean are fenugreek and Egyptian clover.

The reduction in *Orobanche* density per unit area when fenugreek was sown may be due to the growth type of fenugreek, which covers the soil surface and prevents light and other environmental factors required for the germination of *Orobanche* from reaching the weed.

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POTENTIAL OF CINOSULFURON AND CGA152005 SEED TREATMENT FOR CONTROL OF *STRIGA HERMONTHICA* IN UPLAND RICE

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Field trials were conducted in the dry and wet seasons of 1998 at Samaru (11° 11' N, 07° 38' E, 686 m above sea level) in the northern Guinea savanna of Nigeria, to investigate the potential of cinosulfuron and CGA152005 seed treatments on the reaction of upland rice varieties to *Striga hermonthica* (Del.) Benth. Seven varieties of upland rice formed the main plot treatments while four levels each of cinosulfuron at 0.1, 0.2, 0.4 and 0.6 g/l and CGA152005 at 0.008, 0.016, 0.032 and 0.064 g/l, as well as two no herbicide treatments of dry sowing and distilled water-soaked planting were assigned to the subplots. The experiment was laid out in a split plot design and replicated three times. The resistant varieties FARO 40 and WAB 56-50 did not support *Striga* emergence and also produced grain yields which were the maximum, or comparable to the maximum. FARO 11, a susceptible variety, produced high grain yields in spite of support for early, high *Striga* emergence. In spite of delayed emergence of *Striga* on FARO 38 and FARO 48, these varieties, as well as FARO 46 and FARO 45, supported high *Striga* emergence, exhibited high crop reaction scores to *Striga* and produced low grain yields. The seed treatment of upland rice varieties with cinosulfuron at 0.2 to 0.6 g/l and CGA152005 at 0.032 and 0.064 g/l significantly delayed *Striga* emergence compared with the lower rates. After seed treatment with cinosulfuron at 0.6 g/l the susceptible rice variety FARO 38 and the resistant variety WAB 56-50 produced rice grain yields comparable to the maximum obtained with FARO 40 given seed treatment with CGA152005 at 0.064 g/l. The significant interactions of varieties of upland rice and herbicide seed treatments on the number of days to first *Striga* emergence, *Striga* shoot count and crop reaction to *Striga* confirm the differential influence of various concentrations of the herbicide seed treatments on the virulence of *Striga hermonthica* on varieties of upland rice.

Key words: upland rice, *Striga hermonthica*, herbicide treatments

Introduction

Several herbicides that have been used for *Striga* control are applied post-emergence, by which time substantial *Striga* damage to the crop has already occurred (Berner et al., 1995). This suggests that the introduction of chemical control by host seed treatment would alleviate parasitism on the host plant. The use of seed treatments would also be a cost-effective, environmentally friendly method of applying herbicides for the control of root parasites, as it would eliminate the need for application equipment and reduce the quantity of active ingredient required for control (Lagoke et al., 1999).

Acetolactate synthase (ALS) inhibitors such as imidazolinone and sulphonylurea herbicides have been found to be effective in selectively controlling *Striga* in cereals and crop legumes when used as seed treatments. For instance, Abayo et al. (1996) indicated that the seed dressing of maize with imazapyr resulted in about 7 to 9 weeks of protection from attachment. Similarly, the cinosulfuron, CGA152005 and imazaquin seed dressing of cowpea provided control of *S. gesnerioides* and *Alectra vogelii* (Berner et al., 1995). Ransom et al. (1995) indicated that the use of crop seed treatment to delay parasitism by even two weeks had a substantial beneficial effect on maize yield, since the treated crops could get a head start free of parasitic weeds in close proximity to the crop.

Although little research has been conducted on the use of seed treatments to control *Striga* in Nigeria, a few authors have claimed effective control of *Striga* by seed treatment with brine, locust bean extract, *Parkia filicoides*, and *Parkia liglobosa* powder (Konate, 1989; Olaniyan et al., 1991). Good parasitic weed control has been achieved with imazaquin at 1.8 mg/ml (Berner et al., 1994) while Lagoke et al. (1999) also reported the control of *Striga/Alectra* when cowpea seeds were soaked in cinosulfuron at 0.2 and 0.4 g/l and CGA152005 at 0.032 and 0.064 g/l before planting. The efficacy of herbicide seed treatments for the enhanced control of *S. hermonthica* in susceptible and resistant varieties of upland rice was therefore investigated in this study.

Materials and methods

The study was conducted in the dry (Experiment I) and wet (Experiment II) seasons of 1998 at Samaru (11° 11'N, 07° 38'E, 686 m above sea level) in the northern Guinea savanna of Nigeria. The soil of the experimental site was sandy loam with pH 5.3 (H₂O/soil 1:1), organic C 4.2 g/kg, total N 0.73 g/kg and available Bray-1 P 3.31 mg/kg. Exchangeable cations were as follows (cmol^l/kg): Na = 2.12, K = 0.21, Ca = 2.65 and Mg = 1.26. Seven varieties of upland rice, namely FARO 46, FARO 48, FARO 11, FARO 45 and FARO 38 (susceptible), FARO 40 and WAB 56-50 (resistant) formed the main plot treatments, while four levels each of the two herbicides cinosulfuron at 0.1, 0.2, 0.4 and 0.6 g/l and CGA152005 at 0.008, 0.016, 0.032 and 0.064 g/l, as well as two no herbicide treatments, involving soaking in distilled water and dry sowing, were assigned to the subplots. The resistant/tolerant and susceptible varieties were earlier identified for their reaction to *Striga* under both greenhouse and field conditions at Samaru (Adagba, 2000). In the dry season, land preparation was carried out manually after the experimental area was soaked with pipe-borne water using garden hoses. In the wet season, however, the land was mechanically ploughed, disc-harrowed and ridged. The individual plots were marked out and turned into basins. The inner ridges were manually levelled while the outer ridges served as bunds. The dry and wet season trials were planted on the 26th April and 24th June, respectively.

About 20 seeds of the upland rice varieties were initially planted per hill at a spacing of 25 cm × 25 cm. These were later thinned to ten plants/hill at 21 days after sowing (DAS). Fertilizer was applied at the rate of 60-30-30 kg NPK/ha. All the phosphorus and potassium as well as half of the nitrogen were applied at 21 DAS using compound fertilizer (NPK 15-15-15), followed by top dressing with urea at 60 DAS. To ensure uniformity of *Striga* seeds, the fields were on each occasion inoculated with *Striga* seeds immediately before the planting of rice by placing 3.0 g of inoculum mixture in each planting hole, thus ensuring approximately 3000 germinable seeds/hill (Kim, 1994; Magani, 1994). The *Striga hermonthica* seeds were obtained from plants growing on sorghum at Samaru in October/November 1997. Details of *Striga* seed collection and preparation

are described by Kim and Akintunde (1990) and Kim (1994). In the dry season all the plots were watered to field capacity with pipe-borne water every alternate day with garden hoses during the first two months (April/May). Two hoe weedings were carried out at 14 and 28 DAS. Thereafter, hand pulling of emerged weeds of other species was employed till harvest in order to prevent damage to the emerging *Striga* seedlings. The data collected were: number of days to first *Striga* emergence, emerged *Striga* shoot number (infestation), percentage crop infestation, crop reaction score and grain yield. The crop reaction score to *Striga* was based on a 1–9 scale, where (1) was assigned to plots with healthy plants and (9) to those with completely dead plants (Kim, 1991; 1994). All the data were subjected to analysis of variance to test significance and the treatment means were compared using the Duncan Multiple Range Test (DMRT) at the 5% level of probability.

Results

Number of days to first Striga emergence

The number of days to first *Striga* emergence differed significantly among the upland rice varieties and the herbicide seed treatments (Table 1). There was no emergence on FARO 40 and WAB 56-50 and *Striga* emergence was significantly delayed on FARO 38 compared with all the other varieties in Experiment I and on FARO 45 compared with FARO 46 and FARO 38 in Experiment II. In addition FARO 48 had delayed *Striga* emergence compared with FARO 46 and FARO 11 in Experiment I and compared with FARO 46 in Experiment II. Cinosulfuron at 0.4 and 0.6 g/l significantly delayed *Striga* emergence in Experiments I and II compared with its lowest concentration of 0.1 g/l, all rates of CGA152005 and the two no-herbicide control treatments. The number of days to *Striga* emergence with seed treatments of cinosulfuron at 0.2 g/l was also comparable to the maximum and greater than those at the lowest concentration of the herbicide (0.008 and 0.016 g/l) as well as the two no-herbicide controls. CGA152005 at 0.064 g/l significantly delayed *Striga* emergence compared with lower concentrations of the herbicide (0.008 and 0.016 g/l) and the no-herbicide controls in both experiments and also compared with CGA152005 at 0.032 g/l in Experiment I only. The interaction of upland rice varieties and herbicide seed treatments on the number of days to first *Striga* emergence was significant in Experiment I. All the varieties treated with CGA152005 at 0.064 g/l, the distilled water sowing of FARO 48 and FARO 38, all treatments with cinosulfuron except FARO 46, FARO 11 and FARO 45 treated with 0.1 g/l and FARO 11 treated with 0.2 g/l had significantly delayed *Striga* emergence compared with the earliest emergence.

Striga infestation

In Experiment I, apart from FARO 40 and WAB 56-50, the other varieties did not differ significantly in their support for *Striga* emergence at 9 and 12 weeks after sowing (WAS), while FARO 11 supported lower emergence than the maximum at harvest (Table 1). In Experiment II, FARO 48 supported significantly lower *Striga* shoot count at 9 and 12 WAS and at harvest compared with the maximum, while emergence on FARO 45 was only significantly lower at 9 and 12 WAS. FARO 46 also supported lower emergence than the maximum (on FARO 38) at 12 WAS in the trial.

Table 1

Effect of herbicide seed treatments on the number of days to *Striga* emergence and *Striga* shoot count on varieties of upland rice, 1998 dry (I) and wet (II) seasons

Treatments	No. of days to 1 st <i>Striga</i> emergence		<i>Striga</i> shoot count per m ² at					
			9 WAS		12 WAS		Harvest	
	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II
Upland rice varieties (V)								
FARO 46	43.0 ⁴ d	48.2c	5.2a	3.0ab	5.6a	4.7bc	6.6a	7.3a
FARO 11	44.2cd	50.2bc	6.1a	3.4a	5.7a	6.9ab	3.1b	7.9a
FARO 45	46.5bc	55.3a	5.6a	2.1b	4.6a	4.2bc	6.3a	4.8ab
FARO 48	47.4b	52.4ab	5.6a	1.7b	6.0a	2.7cd	5.1ab	3.4bc
FARO 38	50.3a	50.4bc	3.6a	3.0ab	4.6a	8.5a	4.6ab	7.6a
FARO 40	ne	ne	0.0b	0.0c	0.0b	0.0d	0.0c	0.0c
WAB 56-50	ne	ne	0.0b	0.0c	0.0b	0.0d	0.0c	0.0c
SED	1.93	1.06	2.39	0.86	2.13	2.04	1.94	1.75
Herbicide (H) g/l ¹								
Cinosulfuron								
0.1	43.5c	50.0cd	3.4ab	0.8cd	3.7abc	2.4bcd	2.7b	3.3bcd
0.2	52.5ab	57.8ab	1.3b	0.7cd	1.9c	1.7cd	2.3b	2.8cd
0.4	54.7a	60.5a	1.4b	0.15d	1.8bc	0.4d	2.2b	1.2d
0.6	53.7a	59.7a	1.2b	0.0d	1.4c	0.2d	2.0b	0.5d
CGA152005								
0.008	41.0c	43.3e	5.8a	2.4bc	6.8a	7.3a	4.6ab	9.0a
0.016	43.0c	46.9de	5.2a	2.3bc	4.1abc	4.5abc	2.9b	4.8a-d
0.032	41.7c	52.8bcd	5.6a	2.9b	5.4ab	5.9ab	5.2ab	7.6ab
0.064	49.3b	54.6bc	4.3ab	2.0bc	3.8abc	4.0a-d	6.4a	2.5cd
Dry sowing ²	40.3c	46.7de	5.1a	4.6a	5.6a	5.8ab	4.7ab	6.3abc
Distilled water ³	43.1c	46.9de	2.7ab	3.1ab	3.4abc	6.6a	3.7ab	6.5abc
SED	1.8	2.47	1.63	0.83	1.62	1.86	1.56	2.16
Interactions V×H	*	NS	NS	*	NS	*	NS	NS

¹Crop seeds given seed treatment were soaked in herbicide solution for 5 minutes before planting;

²Seeds were not soaked in any liquid before planting; ³Seeds were soaked in distilled water for five minutes before planting; ⁴Means followed by the same letter(s) are not significantly different at the 5% level of probability (DMRT). ne: no emergence; *: Significant at the 5% level of probability; NS Not significant; WAS Weeks after sowing

Seed treatments with all concentrations of cinosulfuron from 0.2 to 0.6 g/l supported significantly lower *Striga* shoot emergence at 9 and 12 WAS and at harvest than the corresponding maximum in the two experiments. At the lowest concentration of 0.1 g/l, cinosulfuron caused a significant reduction in *Striga* shoot count at all the sampling dates in Experiment II and at harvest in Experiment I. CGA152005 at all rates only caused a significant reduction in *Striga* shoot count at 9 WAS, while 0.064 g/l also resulted in lower *Striga* count than the appropriate maximum at harvest (Table 2).

Table 2

Interaction of upland rice varieties and herbicide seed treatments on number of days to *Striga* emergence at Samaru in the dry season of 1998

Treatments	Varieties				
	FARO 46	FARO 11	FARO 45	FARO 48	FARO 38
Herbicides g/l ¹					
Cinosulfuron					
0.1	43.7 ⁴ g-q	36.3opq	39.0 i-q	51.7b- i	45.0f-p
0.2	47.7d-m	41.3j-q	53.0b-g	53.7b-f	55.3b-e
0.4	50.3c-k	47.0e-m	61.3b	53.0b-g	57.0bcd
0.6	52.3b-h	47.3d-m	77.7a	54.7b-f	55.0b-e
CGA152005					
0.008	34.0q	41.0k-q	36.7opq	40.0l-q	42.7h-q
0.016	36.3opq	43.0h-q	38.0m-q	41.3j-q	51.0c-j
0.032	37.3n-q	42.0 i-q	39.3l-q	43.7g-q	55.7b-e
0.064	46.3e-o	58.7bc	47.3d-n	46.3e-o	54.0b-f
Dry sowing ²	42.0 i-q	42.3 i-q	35.0pq	40.3l-q	41.7j-q
Distilled water ³	39.7l-q	43.0h-q	37.7m-q	49.0c- l	46.0e-o
SED			4.05		

¹Crop seeds given seed treatment were soaked in herbicide solution for 5 minutes before planting;

²Seeds were not soaked in any liquid before planting; ³Seeds were soaked in distilled water for five minutes before planting; ⁴Means followed by the same letter(s) are not significantly different at the 5% level of probability (DMRT)

Table 3

Interaction of upland rice varieties and herbicide seed treatments on the *Striga* shoot count at 9 weeks after showing at Samaru in the wet season of 1998

Treatments	Varieties						
	FARO 46	FARO 11	FARO 45	FARO 48	FARO 38	FARO 40	WAB 56-50
Herbicides g/l ¹							
Cinosulfuron							
0.1	3.3 ⁴ b-g	0.0g	0.3g	0.0g	2.0c-g	0.0g	0.8g
0.2	0.0g	1.0efg	0.3g	1.0efg	2.3c-g	0.0g	0.0g
0.4	0.0g	0.0g	0.3g	0.0g	0.0g	0.0g	0.0g
0.6	0.0g	0.0g	0.0g	0.0g	0.0g	0.0g	0.0g
CGA152005							
0.008	1.3efg	5.0b-g	2.7b-g	4.7b-g	3.3b-g	0.0g	0.0g
0.016	6.3b-e	1.3efg	3.0b-g	1.7d-g	4.0b-g	0.0g	0.0g
0.032	6.0b-f	3.7b-g	3.7b-g	2.7b-g	4.0b-g	0.0g	0.0g
0.064	3.3b-g	3.3b-g	0.7fg	2.3c-g	4.0b-g	0.0g	0.0g
Dry sowing ²	7.3bc	16.0a	3.0b-g	3.3b-g	2.7b-g	0.0g	0.0g
Distilled water ³	2.0c-g	3.3b-g	7.0bc	1.7d-g	8.0b	0.0g	0.0g
SED				2.18			

¹Crop seeds given seed treatment were soaked in herbicide solution for 5 minutes before planting;

²Seeds were not soaked in any liquid before planting; ³Seeds were soaked in distilled water for five minutes before planting; ⁴Means followed by the same letter(s) are not significantly different at the 5% level of probability (DMRT)

The interaction of upland rice varieties and herbicide seed treatments was significant on *Striga* shoot emergence at 9 and 12 WAS in Experiment II, in the wet season of 1998. At 9 WAS the maximum *Striga* shoot emergence was observed after the dry sowing of FARO 11 (Table 3). With the exception of FARO 46 and FARO 45 after treatment with CGA152005 at 0.016 g/l and 0.032 g/l or dry sowing and of FARO 38 soaked in distilled water before sowing, seed treatments with all concentrations of the two herbicides and the no-herbicide control treatments on all varieties supported *Striga* shoot emergence that was comparable to the least. At 12 WAS in Experiment II the treatment combinations of CGA152005 at 0.008 g/l on FARO 11 and distilled water soaked planting on FARO 38 resulted in significant *Striga* shoot emergence (Table 4).

Striga incidence (% crop stand infestation)

In addition to FARO 40 and WAB 56-50, which did not support any infestation, FARO 11, FARO 45 and FARO 48 had lower *Striga* incidence compared with the maximum observed on FARO 38 in Experiment II (Table 5), while FARO 48 also had lower *Striga* incidence than FARO 46. In this experiment, seed treatment with the highest concentrations of the two herbicides (cinosulfuron at 0.6 g/l and CGA152005 at 0.064 g/l) resulted in lower *Striga* incidence than the no-herbicide control seeds soaked in distilled water before planting.

Table 4

Interaction between upland rice varieties and herbicide seed treatments on the *Striga* shoot count at 12 weeks after showing at Samaru in the wet season of 1998

Treatments	Varieties						
	FARO 46	FARO 11	FARO 45	FARO 48	FARO 38	FARO 40	WAB 56-50
Herbicides g/l ¹							
Cinosulfuron							
0.1	9.3 ⁴ bc	1.3c	1.3c	1.7c	3.3c	0.0c	0.0c
0.2	1.7c	2.7c	0.3c	0.7c	6.3bc	0.0c	0.0c
0.4	0.3c	0.7c	0.0c	1.3c	0.3c	0.0c	0.0c
0.6	1.3c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c
CGA152005							
0.008	1.0c	25.0a	5.3bc	3.7c	16.0ab	0.0c	0.0c
0.016	6.3bc	3.3c	5.3bc	5.0bc	11.7bc	0.0c	0.0c
0.032	8.7bc	5.3bc	5.7bc	10.0bc	11.3bc	0.0c	0.0c
0.064	1.7c	8.7bc	5.0bc	1.3c	11.7bc	0.0c	0.0c
Dry sowing ²	9.7bc	16.3ab	9.0bc	3.7c	2.0c	0.0c	0.0c
Distilled water ³	7.3bc	6.3bc	10.0bc	0.0c	22.7a	0.0c	0.0c
SED				4.92			

¹Crop seeds given seed treatment were soaked in herbicide solution for 5 minutes before planting;

²Seeds were not soaked in any liquid before planting; ³Seeds were soaked in distilled water for five minutes before planting; ⁴Means followed by the same letter(s) are not significantly different at the 5% level of probability (DMRT)

Crop reaction score

FARO 40 in Experiment I and WAB 56-50 in Experiments I and II had the lowest crop reaction scores compared with the appropriate maxima (Table 5). Similarly, FARO 11 and WAB 56-50 in Experiment I, and FARO 46, FARO 11 and FARO 45 in Experiment II also exhibited lower crop reaction scores than the appropriate maxima. The herbicide seed treatments also influenced crop reaction scores significantly in both the experiments. Cinosulfuron at concentrations of 0.2 g/l to 0.6 g/l and CGA 152005 at 0.064 g/l in Experiment I and cinosulfuron at 0.4 g/l in Experiment II caused lower crop reaction scores compared with the appropriate maxima.

The interaction of upland rice varieties and the herbicide seed treatments on the crop reaction score was significant in Experiment II (Table 6). For FARO 48 this interaction was lower than the maximum with or without seed treatments. This was also true of all the remaining varieties when treated with CGA152005 at 0.032 and 0.064 g/l, of FARO 46, FARO 11 and FARO 38 with seed treatments of cinosulfuron at 0.2 to 0.6 g/l and of FARO 45 with all doses of cinosulfuron.

Table 5

Effect of herbicide seed treatments on percentage crop infestation (%), crop reaction score and rice grain yield

Treatments	% Crop infestation		Crop reaction score		Grain yield kg/ha	
	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II ³
Varieties (V)						
FARO 46	43.5 ⁴ a	14.0ab	4.9a	3.0c	2147c	1310c
FARO 11	32.7a	11.1bc	4.0b	3.1c	2515c	1361c
FARO 45	42.5a	9.5bc	4.4ab	3.2c	2538c	1337bc
FARO 48	44.1a	6.8c	4.4ab	4.2a	2639c	1616abc
FARO 38	39.3a	17.4a	4.3ab	3.8b	2777c	1797ab
FARO 40	0.0b	0.0d	1.7d	2.6d	5415a	1805ab
WAB 56-50	0.0b	0.0d	2.7c	2.3d	3760b	2002a
SED	7.57	2.33	0.40	0.32	405.0	294.9
Herbicides (H) g/l ¹						
Cinosulfuron						
0.1	30.0	5.6cd	3.8a-c	3.1ab	2876	1662ab
0.2	29.0	10.1abc	3.7bc	3.1ab	3210	1695ab
0.4	30.4	6.4abcd	3.3cd	2.8b	3363	1827a
0.6	20.6	3.0d	2.7d	3.2ab	3231	1947a
CGA152005						
0.008	25.4	11.5ab	3.9a-c	3.2ab	2820	1187b
0.016	27.0	8.8abc	4.0abc	3.1ab	3184	1375ab
0.032	31.0	10.9abc	3.8a-c	3.4a	3237	1453ab
0.064	28.7	5.9bcd	3.2cd	3.2ab	3892	1785ab
Dry sowing ²	34.0	9.9abc	4.5a	3.4a	3482	1622ab
Distilled water ³	32.4	12.1a	4.4ab	3.1ab	2834	1487ab
SED	9.18	2.86	0.38	0.25	405.3	260.0
Interactions V × H	NS	NS	NS	*	NS	*

For legend see Table 4

Table 6

Interaction of variety and herbicide seed treatments on the crop reaction score at 9 weeks after showing at Samaru in the wet season of 1998

Treatments	Varieties				
	FARO 46	FARO 11	FARO 45	FARO 48	FARO 38
Herbicides g/l ¹					
Cinosulfuron					
0.1	6.0 ⁴ ab	6.3ab	5.3bc	3.3e	6.0ab
0.2	5.3bc	3.7e	5.0bcd	3.3e	4.3cde
0.4	4.3de	3.3e	3.7e	3.3e	4.3cde
0.6	4.0de	3.0e	3.7e	3.0e	3.3e
CGA152005					
0.008	6.0ab	6.7a	6.3ab	5.0bcd	6.7a
0.016	6.3ab	6.7a	5.7ab	5.3bc	6.0ab
0.032	5.0bcd	4.3cde	3.3e	3.3e	5.0bcd
0.064	4.3cde	3.7e	4.0de	3.0e	3.7e
Dry sowing ²	6.7a	6.0ab	6.3ab	5.0bcd	6.0ab
Distilled water ³	6.7a	6.7a	5.7ab	5.0bcd	6.7a
SED		0.54			

For legend see Table 4

Rice grain yield

The grain yield of upland rice varieties differed significantly in both experiments (Table 5). In Experiment I, FARO 40 had the highest grain yield, while WAB 56-50 also produced significantly higher grain yield than the rest of the varieties, which all produced similar yields. In Experiment II, WAB 56-50 produced significantly higher grain yield compared with FARO 46, FARO 11 and FARO 45, while the yields of FARO 38 and FARO 40 were also higher than FARO 46. The herbicide seed treatments also had a significant effect on the grain yield of upland rice varieties in Experiment II. Cinosulfuron at 0.4 and 0.6 g/l resulted in higher grain yield of upland rice than CGA152005 at 0.008 g/l. The interaction of upland rice varieties and the herbicide seed treatments on the grain yield of rice was significant in Experiment II. FARO 38 and WAB 56-50 treated with cinosulfuron at 0.6 g/l produced grain yields comparable to the maximum produced by FARO 40 with seed treatment of CGA152005 0.064 g/l before planting (Table 7). In FARO 38 treatment with cinosulfuron at 0.6 g/l caused higher grain yield than the lower concentrations, CGA152005 at 0.008 to 0.032 g/l, and the no-herbicide distilled water control. Similarly, the treatment of FARO 40 with CGA152005 at 0.064 g/l caused higher grain yields than all the other rates of the two herbicides, as well as the no-herbicide treatments. WAB 56-50 seeds treated with cinosulfuron at 0.6 g/l produced higher grain yield than all the other concentrations of the two herbicides.

Table 7

Interaction of upland rice varieties and herbicide seed treatments on the rice grain yield in Experiment II at Samaru, 1998

Treatments	Varieties						
	FARO 46	FARO 11	FARO 45	FARO 48	FARO 38	FARO 40	WAB 56-50
Herbicides g/l ¹							
Cinosulfuron							
0.1	1081 ^d e-j	2224c-l	1054e-j	1397d-j	1454d-j	572ij	1274d-j
0.2	1312d-j	1209d-j	1255d-j	2309c-l	1684d-j	1344d-j	1687d-j
0.4	1460d-j	2373b-g	1299d-j	2886bcd	1640d-j	1681d-j	1693d-j
0.6	1551d-j	1326d-j	1308d-j	2886bcd	3788abc	2252c-l	3913ab
CGA152005							
0.008	1266d-j	951f-j	1209d-j	466j	1200d-j	846f-j	1296d-j
0.016	1310d-j	1053e-j	1358d-j	670g-j	1204d-j	1230d-j	1390d-j
0.032	1315d-j	1280d-j	1409d-j	936f-j	1841d-j	1818d-j	1601d-j
0.064	1405d-j	1430d-j	1694d-j	1513d-j	2283c-l	4775a	1815d-j
Dry sowing ²	1162d-j	844f-j	1527d-j	1557d-j	2298c-l	1197d-j	2770b-e
Distilled water ³	1243d-j	921f-j	1251d-j	1539d-j	582hij	2333b-h	2541b-f
SED			688.0				

For legend see Table 4

Discussion

FARO 40 and WAB 56-50 consistently exhibited resistance to *S. hermonthica* in all the experiments. These varieties did not support *Striga* emergence, exhibited low crop reaction scores and also produced grain yields that were at least comparable to the maximum. Similar observations on the reactions of the two varieties to *Striga* in the field and of FARO 40 in the greenhouse were reported by Adagba (2000), who noted that WAB 56-50 supported very low infestation of *Striga* in greenhouse pot trials. The two varieties were observed to stimulate low *Striga* seed germination in the laboratory (Lagoke pers. comm.). Ransom et al. (1996) indicated that the superior grain yields of resistant varieties of maize compared with the susceptible varieties were related to the delayed appearance or total absence of *Striga* parasitism in the resistant varieties. In sorghum, Berner et al. (1995) also observed large increases in productivity when parasitism by *Striga hermonthica* was delayed. On average the grain yield of FARO 40 and WAB 56-50 was twice that of the susceptible varieties. In maize, Kim and Adetimirin (1997) observed that a tolerant variety produced 2.5 times the grain yield of a susceptible variety.

FARO 38, FARO 46, FARO 45, FARO 48 and FARO 11 supported high *Striga* shoot count, a high number of flowering *Striga* and high *Striga* incidence in spite of the delayed emergence of *Striga* on FARO 38, FARO 45 and FARO 48. These varieties also exhibited high crop reaction scores resulting in low grain yields, thus indicating susceptibility to *S. hermonthica*. Riches et al. (1996) also reported that susceptible rice varieties produced stunted plants which did not flower at all and that little biomass was harvested compared to the resistant varieties.

In the present study, cinosulfuron at 0.2 to 0.6 g/l and CGA152005 at 0.064 g/l consistently led to delayed or lower *Striga* emergence in Experiment II compared with the no-herbicide distilled water treatment. The cinosulfuron seed treatments also reduced the *Striga* shoot count on rice plants in Experiment I. This might possibly be due to the fact that in Experiment II, the moisture was adequate for the distribution of the herbicide solution around the seedling rhizosphere, which also enhanced the toxicity of the herbicides. Ransom and Odhiambo (1995) also indicated that the rainfall pattern can significantly affect the timing and intensity of *Striga* parasitism. Cinosulfuron at 0.4 and 0.6 g/l resulted in higher rice grain yields than CGA152005 at 0.008 g/l. The high grain yields obtained might partly be due to the effective reduction of the parasitic weeds by the herbicide seed treatments, especially in the resistant/tolerant varieties. Lagoke et al. (1999) earlier reported that seed treatments of cowpea with cinosulfuron at 0.2 and 0.4 g/l and CGA152005 at 0.032 and 0.064 g/l resulted in higher cowpea grain yield than the lower concentrations and the no herbicide control. The delayed, reduced *Striga* emergence was attributed to the better persistence of the herbicides and/or the phytotoxicity of the seed treatments on the parasitic weed (Abayo et al., 1996; Shinggu, 1998), while the mechanism of control has also been attributed to the post-attachment mortality of the parasite with no reduction in parasitic seed germination (Berner et al., 1995).

The resistant varieties and the higher concentrations of the two herbicides produced superior grain yield when compared with the susceptible varieties and the lower concentrations of the two herbicides. This is obviously related to the delay in *Striga* parasitism in the resistant varieties and to the higher concentrations of the two herbicides, as the final levels of parasitism were similar, as indicated by *Striga* and crop parameters. Berner et al. (1995) earlier indicated large increases in productivity with delayed parasitism in both maize and sorghum. The interaction of upland rice varieties and herbicide seed treatments was significant for number of days to first *Striga* emergence, *Striga* incidence and crop reaction score, suggesting that the herbicide seed treatments differentially influenced the response of upland rice varieties to *S. hermonthica*. It was apparent that *Striga* parasitism was ameliorated on the susceptible rice variety FARO 38 by preplanting seed treatment with cinosulfuron at 0.6 g/l, with the consequent production of a yield comparable to the maximum. Even in the resistant varieties cinosulfuron seed treatment offered protection to WAB 56-50, while CGA152005 at the highest concentration caused the highest production in FARO 40. This therefore indicates that the combination of resistance/tolerance with seed treatments to improve yield offers promise for an integrated approach to *Striga* management.

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INFLUENCE OF PLANT GROWTH REGULATORS AND NITROGEN ON *FUSARIUM* HEAD BLIGHT OF WHEAT (*TRITICUM AESTIVUM* L. VAR. KALYSONA)

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A factorial field experiment was conducted on the demonstration plot of the Plateau Agricultural Development Project (PADP), Jos Plateau State in 1996 with three variables, nitrogen (0 or 80 kg/ha), growth regulator (none, ethephon or chlormequat) and inoculation (none, inoculation with macroconidia of *Fusarium graminearum* at anthesis or inoculation 1 week after anthesis). No head blight symptoms developed on plants in the field that year because of the dry conditions. Grain infection by *Fusarium graminearum* was assessed on a *Fusarium* selective medium. Nitrogen and growth regulators had no effect on grain infection. In 1997 and 1998, a 3×3 factorial experiment was conducted on the same site using the same growth regulator treatments and three inoculation treatments (no inoculation, heads inoculated with macroconidia or rows infested with *Fusarium*-colonized corn kernels that produced ascospores). The incidence of spikelet infection ranged from 2–4% in non-inoculated treatments to 7–25% in inoculated treatments. The incidence of seed infection ranged from 12–31% in non-inoculated treatments to 74–85% in inoculated treatments. Both growth regulators and inoculation treatments had significant effects ($P=0.05$) on spikelet infection and interacted with each other. These data suggest that growth regulators and nitrogen do not change the inherent susceptibility of wheat heads to *F. graminearum*, but dwarfed plants may be subject to higher inoculum doses because they are closer to ejected ascospores.

Key words: growth regulator, nitrogen, *Fusarium graminearum*, wheat

Introduction

One of the most important diseases of wheat (*Triticum aestivum* L.) in northern Nigeria is *Fusarium* head blight or head scab caused by *Gibberella zeae* (Schwein.) Petch. (anamorph = *Fusarium graminearum* Schwabe) (Anaso et al., 1984). The fungus infects flowering heads of wheat during November to December, colonizes developing wheat seeds, and produces mycotoxins, like deoxynivalenol and zearalenone (Sutton, 1982; Giha, 1988). Cultural practices have been shown to influence the incidence of *Fusarium* head blight (FBH). Significantly higher infection and mycotoxin levels were found in wheat planted after corn than in wheat planted after millet (Teich, 1987). Recent research in Quebec has focused on intensive cereal management through the use of nitrogen and growth regulators (Ayoub et al., 1994). Reports by Martin et al. (1991) and Rowaished (1981) indicated that supplemental nitrogen and the plant growth regulator, ethephon increased the incidence of natural infection by *F. graminearum* in wheat cultivars. Others found that head blight and mycotoxin levels were not affected by the level of applied nitrogen but were greater when

the preceeding crop was maize (Teich and Hamilton, 1985). Both ethephon and chlormequat increased the incidence of spikelet infection, but only in treatments inoculated with infested corn (Fauzi and Paulitz, 1994). The objective of the present work was to determine the effect of growth regulators on plant architecture and microclimate, thus exerting an indirect influence on inoculum production and dispersal.

Materials and methods

Fungal isolate

A single-spore isolate of *F. graminearum* (isolate 7), collected from Hadeija, Kano State in 1996, tested for pathogenicity and deposited at the National Cereals Research Institute (NCRI) Badeggi, Niger State, Nigeria was used in the 1996 field trial. Isolate 7 produced sparse perithecia on potato dextrose agar (PDA) after 2–3 months in culture. Another isolate of *F. graminearum*, DAOM 178148, from the NCRI, was used in the 1997 and 1998 field trials because it produced more perithecia in culture. Both isolates are, however, virulent on wheat (var. Kalysona).

Preparation of inoculum

Macroconidia for the inoculation of wheat spikes in the field experiments were produced in carboxymethyl cellulose medium (CMC) (Cappellini and Peterson, 1995). A plug 4 mm in diameter, taken from the margin of a 5-day old culture of *F. graminearum* on PDA was placed in 500 ml of CMC medium in a flask and incubated on a rotary shaker (100 rpm) for 7 days at 24°C with 16 hours per day of combined fluorescent and incandescent lighting. Conidia were harvested from the flask by filtration through two layers of sterile cheesecloth. The conidial density was determined with a haemocytometer. Ascospore inoculum was produced on maize kernels. After 500 g maize was autoclaved in 1-litre glass jars for 45 minutes on two consecutive days, 10 ml macroconidial suspension was placed in each jar, which was then sealed with a canning lid in which two holes 6 mm in diameter had been punched. A filter disk 70 mm in diameter was placed on the inside of the lid to permit air exchange while maintaining sterility. The jars were shaken to distribute the spores, then incubated at 20°C under long-wave UV and fluorescent light for about 2 months.

Inoculum density test

The volume of spore suspension that would be deposited on a wheat head using an artist's airbrush at 103 Kpa pressure was determined using 10 wheat heads which were each uniformly sprayed for 5 seconds at a distance of 5 cm with a known concentration of conidia, after which each head was placed in a flask with 99 ml of sterile water. The flasks were agitated on a rotary shaker for 3 minutes and the suspension was dilution-plated on PDA amended with 100 µg/ml of chloramphenicol (Fauzi and Paulitz, 1994). The test established that approximately 70 µl suspension was deposited on each head.

Field experiment, 1996

The effects of nitrogen fertilization and growth regulator application on *Fusarium* head blight were studied in 1996 in inoculated field plots of the wheat variety Kalysona on a sandy loam soil at the Plateau Agricultural Development Project, Jos.

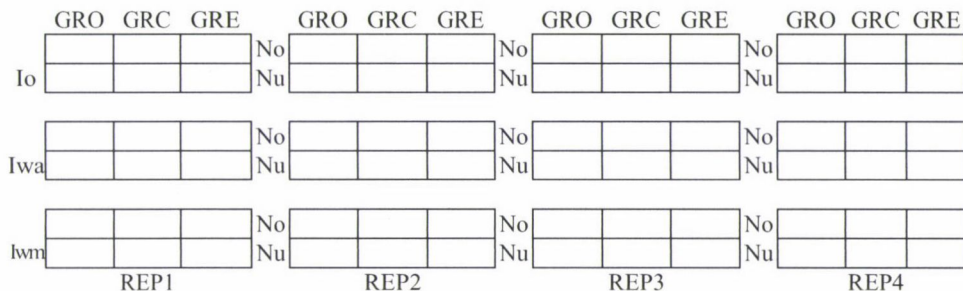
The experiment was arranged as a $3 \times 3 \times 2$ factorial in a randomised split-plot design, with four replicates (Fig. 1). The main plots were inoculated 1 or 2 weeks after anthesis with macroconidia or ascospores; non-inoculated plots served as the control. The sub-plots were growth regulator treatments: no treatment and treatment with chlormequat or ethephon. The sub-sub plots were nitrogen treatments: no nitrogen and urea. Each plot was 3.8 m long, with 11 rows spaced 10 cm apart. Each main plot was separated by a 9-m border plot of Kalysona and each block was separated by a 3-m bare walk-way. The plots were seeded on 7th May by hand. One week after

seeding, half the plots were hand-fertilized with nitrogen in the form of urea at 80 kg N/ha. When the wheat reached Zadoks growth stage (ZGS) 31 (Zadoks et al., 1974) the chlormequat plots were sprayed at a rate of 1.2 kg a.i./ha. When the plants in the ethephon plots reached ZGS 45, they were sprayed with 480 g a.i./ha of ethephon. Both growth regulators were applied with a backpack sprayer. Plots not treated with growth regulators served as the control. On 9th July, 1 week after anthesis, the heads of the plants in one treatment were inoculated in the morning with macroconidia of *F. graminearum* using spray bottles filled with a suspension of 6×10^5 conidia per millilitre. In another treatment, the heads were inoculated 2 weeks after anthesis, also in the morning, while non-inoculated plots served as the control. All plots were irrigated with a lawn sprinkler for 15 minutes every 2 hours during the day for 2 weeks after inoculation.

The incidence of grain infection by *F. graminearum* was assessed by plating 100 seeds harvested from each plot on selective medium developed to induce rapid sporulation of *Fusarium* spp. The medium contained agar (20 g/l), potato dextrose broth (12 g/l), pentachloronitrobenzene (1.5 g/l) and chloramphenicol (0.5 g/l). The seeds were surface-disinfected for 6 minutes in 0.5% sodium hypochlorite, rinsed three times in sterile distilled water, blotted on sterile paper towels, and plated 10 to a plate. The plates were incubated at room temperature (22–24 °C) for 5 days. The *Fusarium* colonies originating from the seeds sporulated rapidly, and *F. graminearum* was identified by the carmine-red colour of the colony and the shape and size of the macroconidia.

Field experiment, 1997 and 1998

Similar field experiments were conducted at the Central Experimental Farm of Agriculture in Hadeija, Nigeria where an automatic misting system was available to create more favourable conditions for disease. A different isolate of *F. graminearum* (DAOM 1768148) was used, one that produced perithecia more profusely than the isolate used in 1996. The nitrogen applications (sub-sub plots) were omitted in 1997/1998 since the nitrogen level in the field was already high (>40 kg N/ha). The experiments were 3×3 factorials arranged in a split-plot design with four replicates (blocks). All the plots were 3 m long, with eight rows 22 cm apart. The main plots were inoculated with macroconidia or ascospores; non-inoculated plots served as the control. The sub-plots were growth regulator treatments: no treatment and treatment with chlormequat or ethephon. The main plots were separated by a 3-m border of cv. Fielder, and the blocks were separated by a 3-m walkway. Wheat was seeded on 15th and 24th May in 1997 and 1998, respectively. Weeding was done by hand. The growth regulators were applied as in the 1996 experiment. Macroconidia inoculum was applied with a backpack sprayer at anthesis on 14th July 1997 and 25th July 1998 as previously described. On 10th June 1997 and 19th June 1998, when the wheat was approximately 15 cm high, 500 g *Fusarium*-colonized corn kernels was applied to the ascospore inoculation treatments.



Io: No inoculation (This is subject to randomization); Iwa: Inoculation with ascospores; Iwm: Inoculation with macroconidia; GRO: No growth regulator; GRC: Growth regulator (chlormequat); GRE: Growth regulator (Ethephon); No: No nitrogen; Nu: Nitrogen urea; Each plot was 3.8 m \times 2 m in size; Main plots were separated by 9 m border plot; Replicates were separated by a 3 m walkway

Fig. 1. Trial lay-out

Two weeks later the corn kernels were covered with purple-black perithecia containing mature ascospores. After anthesis and until the disease symptoms appeared, the plots were irrigated with an automatic mist system, which turned on for 30 seconds every 5 minutes from 6 a.m. to 8 p.m. Disease was assessed using a Standard Evaluation System scale (0–9) (IRRI, 1988) by counting the number of infested spikelets per head on 50 heads randomly chosen from the middle of each plot. Grain infection was assessed by plating 100 seeds from each plot on *Fusarium* selective medium, as previously described.

Statistical analysis

The data were analysed with analysis of variance (ANOVA) procedures to determine the significance of treatment effects and their interactions (SAS Institute, 1985). A protected least significant difference (LSD) test at the 0.05 level was carried out to compare treatments. Orthogonal contrast tests were used on data from the 1997 and 1998 field trials.

Results

During the 2-week period after inoculation in the November 1996 trial, there was no mist, with a high temperature of about 30°C during the second week. No symptoms of *Fusarium* head blight were observed in the field, but grain infection ranged from 2–20% in treatments inoculated with macroconidia. Only the inoculation treatments had a significant effect on grain infection (Table 1); there were no significant interactions between the variables. There was no grain infection in the non-inoculated treatments; 10% infection occurred in the treatment inoculated at anthesis, and 8% infection in the treatment inoculated 1 week after anthesis. Nitrogen showed no significant main effect ($P = 0.93$) or interactions. Growth regulator treatment had no significant main effect ($P = 0.06$).

The 1997 and 1998 field trials were more favourable for head blight than 1996; the atmosphere was misty with an optimum temperature of 25–27°C. Disease severity was thus much higher than in 1996. The incidence of spikelet infection ranged from 2–4% in non-inoculated treatments to 7–22% in inoculated treatments. The incidence of grain infection ranged from 12–31% in non-inoculated to 74–85% in inoculated treatments in the two years. Both growth regulators and inoculation treatments had significant effects on spikelet infection and interacted with each other (Tables 2 and 4). In plots treated with *Fusarium*-colonized corn, where the inoculum source was ascospores, the incidence of scabby spikelets was higher in treatments sprayed with chlormequat or ethephon than in treatments with no growth regulator. However, no significant differences between the growth regulator treatments were seen in non-inoculated plots or plots inoculated with macroconidia. Both inoculation and growth regulator treatments also had a significant effect on the incidence of grain infection in 1997/1998, but the interaction between the two variables was not significant. When compared with the non-treated control, chlormequat significantly increased grain infection both in non-inoculated plots and in plots treated with *Fusarium*-colonized corn (ascospores) (Tables 3 and 5). Growth regulators had no effect on grain infection in plots where the heads were directly inoculated with macroconidia.

Table 1

Effect of growth regulators and inoculation treatment on grain infection of wheat (var. Kalysona) by *Fusarium graminearum* in the 1996 field trial

Inoculation treatment	Incidence of grain infection (%)		
	Control	Ethephon	Chlormequat
Water at anthesis	0	0	0
Macroconidia at anthesis	9.1 ^{a*}	7.4 ^a	11.7 ^a
Macroconidia 1 week after anthesis	6.1 ^a	6.5 ^a	9.8 ^a

*Treatments within a row followed by the same letter are not significantly different according to a protected least significant difference test, $P=0.05$.

Table 2

Effect of growth regulators and inoculation treatment on spikelet infection of wheat (var. Kalysona) by *Fusarium graminearum* in the 1997 field trial

Inoculation treatment	Incidence of spikelet infection (%)		
	Control	Ethephon	Chlormequat
Non-inoculated	1.6 ^{a*}	1.6 ^a	1.6 ^a
Ascospore inoculation from perithecia on colonized corn	9.4 ^a	13.4 ^b	13.6 ^b
Inoculation of heads at anthesis with macroconidia	13.1 ^a	10.7 ^a	13.6 ^a

*Treatments within a row followed by the same letter are not significantly different according to orthogonal contrasts, $P=0.05$.

Table 3

Effect of growth regulators and inoculation treatment on grain infection of wheat (var. Kalysona) by *Fusarium graminearum* in the 1997 field trial

Inoculation treatment	Incidence of grain infection (%)		
	Control	Ethephon	Chlormequat
Non-inoculated	11.3 ^{a*}	16.2 ^a	22.6 ^a
Ascospore inoculation from perithecia on colonized corn	75.8 ^a	82.2 ^b	87.1 ^b
Inoculation of heads at anthesis with macroconidia	75.2 ^a	77.4 ^a	80.6 ^a

*Treatments within a row followed by the same letter are not significantly different according to orthogonal contrasts, $P=0.05$.

Table 4

Effect of growth regulators and inoculation treatment on spikelet infection of wheat (var. Kalysona) by *Fusarium graminearum* in the 1998 field trial

Inoculation treatment	Incidence of spikelet infection (%)		
	Control	Ethephon	Chlormequat
Non-inoculated	1.7 ^{**}	1.6 [*]	1.7 [*]
Ascospore inoculation from perithecia on colonized corn	9.8 [*]	13.7 [*]	13.9 [*]
Inoculation of heads at anthesis with macroconidia	13.3 [*]	10.9 [*]	13.9 [*]

*Treatments within a row followed by the same letter are not significantly different according to orthogonal contrasts, $P=0.05$.

Table 5

Effect of growth regulators and inoculation treatment on grain infection of wheat (var. Kalysona) by *Fusarium graminearum* in the 1998 field trial

Inoculation treatment	Incidence of grain infection (%)		
	Control	Ethephon	Chlormequat
Non-inoculated	11.6**	16.8*	23.6*
Ascospore inoculation from perithecia on colonized corn	76.5*	83.5*	88.3*
Inoculation of heads at anthesis with macroconidia	76.3*	78.5*	81.2*

*Treatments within a row followed by the same letter are not significantly different according to orthogonal contrasts, $P=0.05$.

Discussion

Nitrogen failed to affect disease in the field in 1996. Although mist was scarce during the period of inoculation and no visible symptoms developed, it is thought that differences in grain infection would reflect any differences in susceptibility mediated by the nitrogen. The form of nitrogen is also important, as shown in previous studies on other diseases. Wheat fertilized with urea had less head blight than that fertilized with ammonium nitrogen (Teich, 1987).

The application of growth regulators in the field in 1996 had no effect on grain infection by *F. graminearum*, possibly because of the unfavourable conditions prevailing that year. In 1997 and 1998, growth regulators had no effect in the field trials in spray-inoculated plots, when conditions were favourable for disease development because of high misting. In all the head inoculation treatments the macroconidia were applied directly to the head, thus eliminating the effects of growth regulators on the microclimate in the canopy and the position of susceptible heads relative to the inoculum (Martin et al., 1991).

The use of *Fusarium*-colonized corn kernels in the field simulated the natural inoculum in the field more correctly. Ascospores are thought to be more important than macroconidia in the epidemiology of this disease (Sutton, 1982). Ascospores are forcibly discharged during the evening hours in response to falling temperatures and rising relative humidity and can be detected above the wheat canopy during a 3-week period that coincides with anthesis (Stack, 1989). In treatments that used mature perithecia, chlormequat significantly increased both spikelet and grain infection, but not in treatments inoculated with macroconidia. Chlormequat also increased grain infection in non-inoculated plots, which probably received inoculum that had drifted from the inoculated plots. Ethephon also increased spikelet infection in this study.

The difference in disease incidence between treatments in which heads were inoculated with macroconidia and treatments receiving ascospore inoculum from perithecia suggests that plant growth regulators increased head blight by decreasing head height and placing it closer to the inoculum source. Plants treated with chlormequat and ethephon were on average 18 and 12 cm shorter, respectively, than the non-treated plants. Martin et al. (1991) observed that ethephon increased *Fusarium* head blight in field plots with natural inoculum.

Bockman (1968) reported similar results with chlormequat on wheat infected with *F. culmorum* and *Septoria nodorum*, suggesting that one of the causes was the shorter distance that the spores would have to travel to reach leaves and heads. Mesterhazy (1991) showed that dwarf genotypes of wheat were more susceptible to head blight based on natural infection in field plots. Artificial inoculation resulted in no significant differences, suggesting that plant height is a morphological trait that influences natural infection but causes no change in plant resistance. The inoculum potential of ascospores and macroconidia is similar (Stack, 1989), so the difference between the two treatments is probably due to variables in inoculum density and not inoculum potential. Another possibility is that the dwarf habit of wheat treated with growth regulators may influence the microenvironment of crop debris on the soil, leading to greater production of perithecia and ascospores.

It is pertinent to mention that growth regulators such as ethephon and chlormequat increase infection in susceptible cultivars only.

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RESPONSES OF IRANIAN ONION (*ALLIUM CEPA* L.) GERMPLASM TO SEMI-SOLID SALINE MEDIUM

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Onion (*Allium cepa* L.) is regarded as a plant susceptible to salts during seed germination and establishment. This study was conducted to evaluate the tolerance of 10 Iranian onion populations to NaCl at the seed germination and seedling growth stages, to determine critical NaCl concentrations for these populations and to select NaCl-tolerant seedlings. The NaCl concentrations were 0, 0.3, 0.6, 0.9 and 1.2%. The populations differed significantly for percentage of seed germination rate, fresh and dry weights of the seedlings, and the length of radicles and whole seedlings ($p < 0.01$). Significant differences were also observed between the NaCl concentrations ($p < 0.01$). All the NaCl concentration \times population interactions were significant ($p < 0.01$). This experiment indicated that the salt tolerance of various populations differed during germination and seedling growth. Populations 3 and 7 were more tolerant than the other populations. However, within each population, salt-tolerant seedlings were selected for additional studies.

Key words: *Allium cepa*, Iranian onion germplasm, seed germination, seedling growth, NaCl stress, salt-tolerant seedlings, semi-solid saline medium

Introduction

Soil salinity is one of the major impediments to agriculture in arid and semi-arid regions of the world (Akbar and Yabuno, 1974; Ashraf et al., 1986a; 1986b; Ashraf and Waheed, 1990; Epstein, 1985). A biotic approach to overcome the salinity problem has been considered to be the most feasible and economic path and has recently received much attention (Ashraf and Neilly, 1987; Epstein, 1985).

The different strategies adopted by various plant scientists in employing a biological approach to overcome the salinity problem were listed by Ashraf and Waheed (1990). One procedure, which is of prime importance, is the screening of the available local germplasm of a crop for salt tolerance to identify tolerant populations which may, hopefully, sustain a relatively good yield on salt-affected soil (Ashraf and Neilly, 1987; Ashraf and Waheed, 1990).

Onion (*Allium cepa* L.) is one of the major vegetable crops throughout the world and has considerable importance as food (Miyamoto, 1989; Yadav, 1994). Onion, like many other vegetable crops, is sensitive to salinity and may not sustain a good yield under such conditions (Wannamaker and Pike, 1987). Therefore, the screening of available germplasm may provide a relatively tolerant cultivar.

The ability of seed to germinate in saline environments is an essential factor and has been studied to some extent in onion (Miyamoto, 1989; Wannamaker and Pike, 1987). Miyamoto (1989) investigated salt effects on the seed germination, emergence and seedling mortality of 5 onion cultivars in a series of greenhouse experiments at diurnal temperatures of 15 to 25°C. Germination exceeded 80% within 10 d at salinity levels of 20 dS/m or less. Differences in germination between the cultivars were observed initially, but the difference decreased after approximately 1 week. Seedling emergence in a loamy sand medium declined significantly when the salinity of the irrigation water exceeded 4.9 dS/m. This decline was apparently caused by hypocotyl mortality when the cotyledon came into contact with the salts accumulated on the soil surface, not by reduced seed germination (Miyamoto, 1989). Yadav (1994) studied the effects of salinity (0, 4, 8, 12 or 16 dS/m) on the germination and seedling growth of onions. The percentage of germination and seedling growth decreased with increasing salinity (Yadav, 1994). The present study was conducted to examine salt effects on seed germination and seedling growth of Iranian onion germplasm (Iranian onion populations) in a semi-solid saline medium.

Materials and methods

Ten Iranian onion germplasms or Iranian onion populations (Pop-1 to Pop-10) were collected from different areas of Azarshahr in Eastern Azarbijan Province. All the onion populations were long-day and had long storage life.

The research approach of Carlson et al. (1983) was used with the following modifications:

1. The seeds were sterilized by successively adding the following solutions, aspirating each after the indicated time period:
 - a.) 95% ethanol: the seeds were swirled for about 10 sec.
 - b.) 2.5% Chlorox with 0.1% Tween 40: the seeds were swirled in this solution, covered, and allowed to stand for 5 min, then the Chlorox-Tween solution was aspirated.
 - c.) 20 ml sterile distilled water: the seeds were washed three times.
2. The seeds were dried on Watman No. 1 filter papers.
3. Twenty seeds were put on sterile solidified water-agar medium containing 0.0, 0.3, 0.6, 0.9 and 1.2% NaCl (without any other chemicals) in glass jars.
4. For each treatment 5 glass jars were considered as the samples.
5. The cultures were incubated at 20°C with a 16/8 h photoperiod.
6. Germination (defined as percentage of seeds that showed a combined radicle and hypocotyl growth of at least 1 mm) was monitored on the 2nd, 3rd, 4th, 5th, 10th and 15th days.
7. After 30 days, the length and the fresh and dry weights of seedlings, shoots and radicles were measured.

In order to study the effects of NaCl concentration on the seed germination and seedling stages of the ten Iranian onion populations mentioned earlier, a factorial experiment based on a completely randomized design with 5 replications was carried out in the Khalat-Pooshan Research Station of Tabriz University.

Results

Germination experiment

The NaCl concentrations provided a full range of responses (from 0.0 to 100% germination) for the populations under study. Analysis of variance on the data for germination percentages 2nd, 3rd, 4th, 5th and 10th days and for the final germination percentages (15th day) and germination rate showed highly significant ($p < 0.01$) differences between the NaCl concentrations (Table 1). The germination percentage and rate of germination of onion populations exposed to saline media were significantly ($p < 0.01$) lower than the control (no NaCl). Germination was inhibited at NaCl concentrations higher than 0.9% (Figs. 1 and 2). The germination percentages of all the populations in NaCl media increased over time. However, little change in the germination percentages occurred after the 5th day (Fig. 1). The populations showed significant ($p < 0.01$) differences in their ability to germinate on the saline media (Table 1). A comparison of the means of the 10 populations indicated that Pop-3 and Pop-7 had the highest germination percentage and rate of germination. Maximum differences between the populations for germination under salt stress were observed at 0.9% NaCl. Population \times NaCl concentration interactions were also significant for the characteristics under study ($p < 0.01$) (Table 1).

The dendrogram obtained from cluster analysis based on germination responses is given in Fig. 3. Four main clusters were obtained: A) Tolerant: Pop-3 and Pop-7; B) Semi-tolerant: Pop-4 and Pop-10; C): Semi-sensitive: Pop-1, Pop-2 and Pop-9; D) Sensitive: Pop-5, Pop-6 and Pop-8.

Seedling experiment

There were significant differences between the populations and between NaCl concentrations for seedling characters (Table 2) ($p < 0.01$). The seedling fresh and dry weights, seedling shoot and root length, and shoot to root ratio of all the populations decreased as the NaCl concentration increased (Figs. 4, 5 and 6). A comparison of the seedling growth parameters of the populations indicated that Pop-3 and Pop-7 were more tolerant than the other populations. Maximum differences between the populations for seedling traits were observed at 0.9% NaCl. Population \times NaCl concentrations were also significant for the characteristics under study ($p < 0.01$) (Table 2).

In the course of cluster analysis the 10 populations were classified into the following 3 groups on the basis of their seedling growth traits (Fig. 7): A) Tolerant: Pop-3 and Pop-7; B) Semi-tolerant: Pop-1, Pop-2 and Pop-8; C) Sensitive: Pop-4, Pop-5, Pop-6, Pop-9 and Pop-10. Cluster analysis of the 10 populations on the basis of their performance both in germination and seedling experiments resulted in the same three clusters (Fig. 8).

Table 1

Analysis of variance of onion populations for germination percentages and germination rates in response to different NaCl concentrations (mean squares)

Source	2nd day	3rd day	4th day	5th day	10th day	Final ger. ^a	Ger. rate
Population (P)	0.242**	0.162**	0.123**	0.076**	0.084**	0.082**	0.001**
Salt levels (S)	1.180**	1.305**	1.839**	2.227**	2.097**	2.146**	0.004**
P × S	0.016**	0.01**	.025**	0.045**	0.0412**	0.057**	0.0003**
Error	0.003	0.004	0.004	0.003	0.0032	0.0031	0.000031
CV ^a (%)	16.67	15.77	11.55	8.35	8.31	8.18	1.96

** Significant at the 0.01 level of probability; Cv : Coefficient of variation

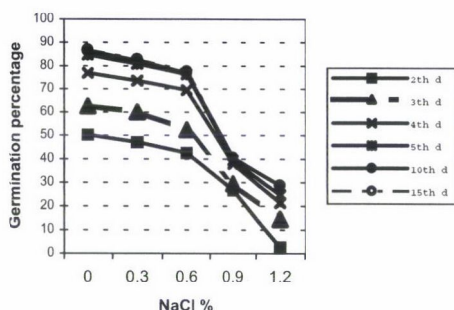


Fig. 1. Mean germination percentage after the 2nd to 15th days in various saline solutions

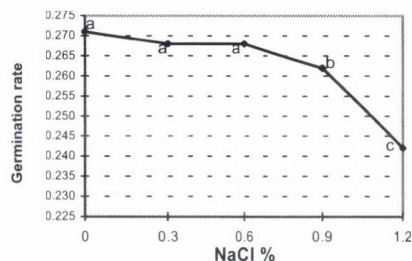


Fig. 2. Mean rate of germination after 5 days in various saline solutions (Duncan's new multiple range test, $p = 0.01$)

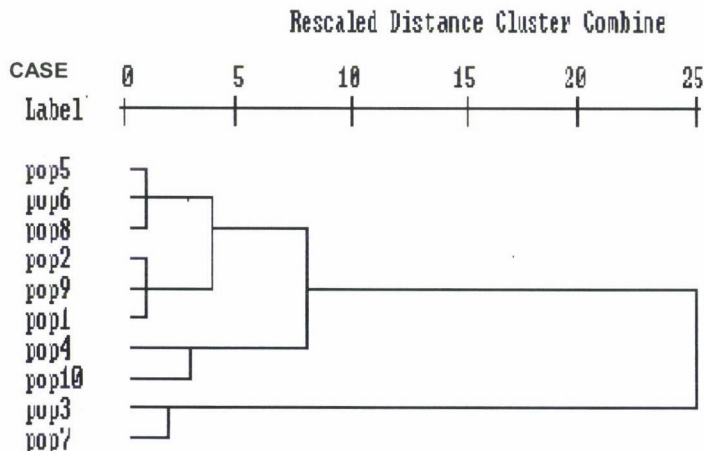


Fig. 3. Dendrogram obtained from cluster analysis (Ward's method) of 10 populations based on differential germination responses across five NaCl concentrations

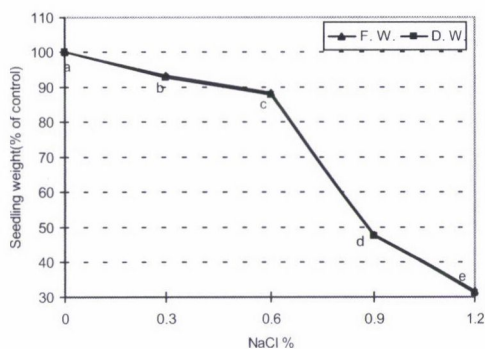


Fig. 4. Mean fresh and dry weights of seedlings after 25 days in various saline solutions (Duncan's new multiple range test, $p=0.01$)

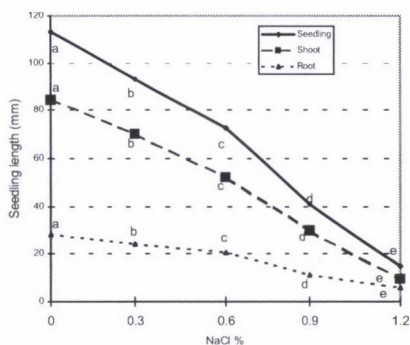


Fig. 5. Mean seedling, shoot, and root length after 25 days in various saline solutions (Duncan's new multiple range test, $p=0.01$)

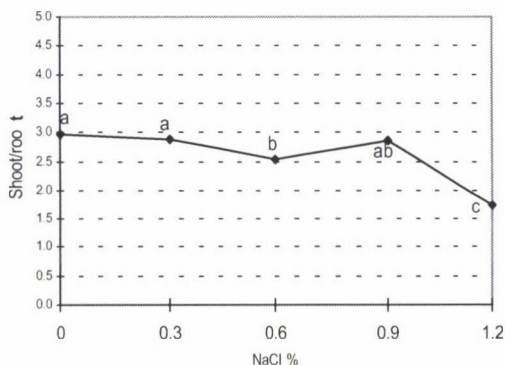


Fig. 6. Mean shoot to root ratio after 25 days in various saline solutions (Duncan's new multiple range test, $p=0.01$)

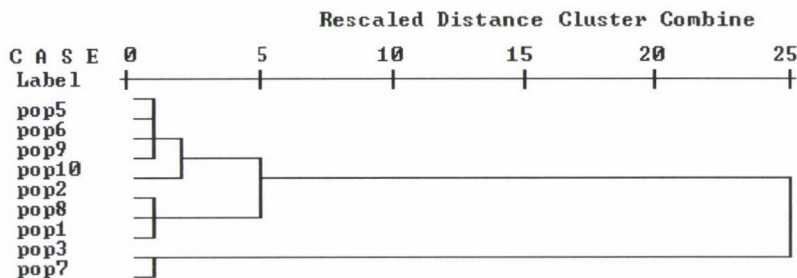


Fig. 7. Dendrogram obtained from cluster analysis (Ward's method) of 10 populations based on differential seedling responses across five NaCl concentrations

Table 2
Analysis of variance of onion populations for seedling traits in response to different NaCl concentrations

Source	F.W. (% of control)	D.W. (% of control)	Seedling length (mm)	Shoot length (mm)	Root length (mm)	S/R
Population (P)	496.45**	452.45**	1191.05**	617**	100.21**	0.491*
Salt levels (S)	27908.192**	2807.59**	46967**	27214.15**	2701.77**	7.23**
P × S	103.335**	104.218**	68.48**	43.57**	6.325**	0.296ns
Error	1.414	2.505	3.55	5.52	1.065	0.12
CV ^a (%)	1.65	2.21	2.82	3.24	5.73	10.74

** Significant at the 0.01 level of probability; ns: non-significant; CV : Coefficient of variation

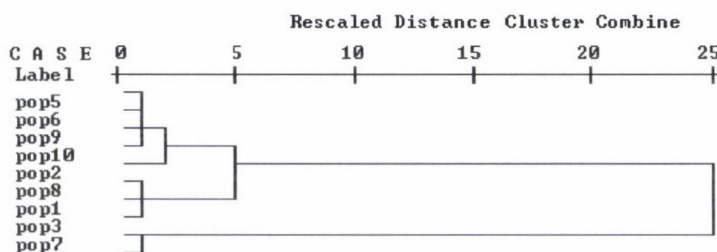


Fig. 8. Dendrogram obtained from cluster analysis (Ward's method) of 10 populations based on differential germination and seedling responses across five NaCl concentrations

Discussion

The existence of genetic variation in salt tolerance is a prerequisite for the development of salt-tolerant cultivars through breeding. To explore such variation and the effect of NaCl on seed germination and seedling growth in Iranian onion populations, 10 landrace populations were investigated at 2 early growth stages, as salt tolerance throughout these two stages is crucial for the establishment of a crop in a saline environment.

Data for different growth parameters (Figs. 3, 7 and 8) clearly show that only two populations (Pop-3 and Pop-7) were categorized as the most NaCl-tolerant among the 10 populations. The range in the responses of onion populations when germinated on semi-solid media containing NaCl indicated that it should be possible to select for salt resistance. A knowledge of the tolerance observed at the germination and seedling stages would be of considerable economic value for crop establishment on salt-affected soils (Ashraf and Neilly, 1987; Ashraf and Waheed, 1990). A reduction in germination percentage, rate of germination and seedling growth parameters

was observed with increasing NaCl concentrations (Figs. 1, 2, 4–6). Maximum differences between the populations for germination and seedling stages under salt stress were observed at 0.9% NaCl for all characteristics. The germination percentages of all the populations in NaCl media was increased over time. However, little change in germination percentages occurred after the 5th day (Figs. 1 and 2). Wannamaker and Pike (1987) found no significant differences in germination percentage between cultivars or salt level for the 0.0 to 25.0 dS/m salt levels. At 30.0 dS/m all cultivars exhibited reduced germination of varying degrees. Miyamoto (1989) indicated that the difference in germination between the cultivars 5 days after seeding was significant, but the difference was not great. Yadav (1994) showed that the percentage of germination and seedling growth decreased with increasing salinity.

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DETERMINATION OF OPTIMUM SPATIAL ARRANGEMENT OF RICE PLANTS IN DIRECT SEEDING METHODS

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Crop stands established by direct seeding methods using row seeder or broadcaster are usually constrained by non-uniform and sub-optimal spatial arrangements. Field investigations were done to identify and evaluate their effect on rice crop production. These investigations revealed that the distance away from neighbouring seedlings had effects on rice yield because there were localized variations in the competition. In an extended attempt to determine the optimal spatial arrangement of rice plants, experimental studies were carried out in lysimeters, mini-lysimeters and pots in a controlled environment. From the experimental results it was made clear that the manipulation of spatial geometry has promising potential for increased crop yield. It is concluded that yield gains can be achieved by mechanized direct seeding that adopts an optimum spatial arrangement.

Key words: crop yield, direct seeding method, large-sized paddy field, plant spacing, *Oryza sativa*

Introduction

Rice (*Oryza sativa*) is a relatively labour-intensive crop. Often about twice as much labour is employed per hectare compared with other grain crops and legumes (Kimura, 1977; Nakamura, 1983). The most obvious way of raising labour productivity is to substitute some alternative form of energy, say mechanization (Koga, 1992; Murugaboopathi, 1992; Yamaji, 1992). Till 1970, most of the rice planting in Japan was done manually and transplanting was predominant (Inoue, 1982). Since then, in order to reduce labour input in rice planting, rice transplanters have been introduced. Although transplanters are in wide use, they still involve considerable labour in nursery seedling raising, staking in the transplanter as well as transplanting. Hence, direct seeding is adjudged to be an alternative way to reduce labour requirements further. Direct seeding is usually done either by a direct seeder in straight rows or by a broadcasting machine. The non-uniform distribution of seeds in the field is a big problem when employing these methods. This non-uniformity ultimately reduces the yield even though increased labour productivity can be achieved. Sub-optimal crop stands produced using a row seeder or broadcaster in large-sized paddy fields in Japan are characterized by both low and high mean plant populations and by an uneven distribution of plants (Yamazaki, 1992; Makiyama, 1994). In an attempt to develop precise direct seeding techniques this study had the following objectives: (i) to determine the optimal intra-row spacing, if any, which can be useful in making row seeding methods more

efficient, (ii) to quantify the effect of sub-optimal plant to plant spacing in precise geometrical arrangements, and (iii) to evaluate and compare different (non)uniform — random spatial arrangements and their influence on crop production under varying crop density conditions, in broadcasting methods.

Materials and methods

Field studies

In Field A, a 1.5 ha large-sized paddy field in Chiba Prefecture, Japan, crop stands of cultivar *Koshihikari* were established with a row seeder in summer 1994. Along an 800 cm track, 225 plants were identified and sampled; their intra-row spacing was measured. In summer 1995, a tractor-driven broadcaster was used in Field B, another 2.14 ha large-sized paddy field near to Field A, to establish crop stands. The number of plants was counted in a 50 × 50 cm cell area and grain yield was calculated at 18 random locations within the field. A description of the fields is available elsewhere (Anbumozhi, 1995).

Experimental studies

Individual experiments were carried out in duplicate in summer 1994 with six different row spacings, three different plant-plant spacings and four different random spatial arrangements; the plants were grown in lysimeters (120 × 120 cm), mini-lysimeters (53 × 12 cm) and pots (25 cm dia). The effect of changes in intra-row spacing on the rice crop yield was studied in lysimeters and mini-lysimeters in summer 1994. The lysimeter experiment included six intra-row spacing treatments of 1, 2, 3, 4, 7 and 10 cm in a 80 cm row. A constant distance of 20 cm was maintained between rows. The above experiment was repeated in the following year with the inclusion of 0.5 cm and 5 cm intra-row spacings and the exclusion of greater spacing intervals.

The effect of three crop densities and plant to plant spacings on growth, performance and production was studied in mini-lysimeters during summer 1994. The crop densities under study on a 1855 cm² area were dense (96 plants), moderate (24 plants) and sparse (8 plants). The plant populations corresponded to 4 cm, 8 cm and 12 cm square grid spacing, as a precise geometrical arrangement. With the total area available remaining constant, each plant thus occupied 19.3, 77.3 and 231.8 cm² in the dense, moderate and sparse densities, respectively.

In order to evaluate the effect of random spatial arrangement on crop yield, studies on crop density and spatial arrangement were conducted in pots during summer 1995. Four spatial patterns were compared under three densities in pots with a surface soil area of 500 cm². The spatial patterns established were Random I, Random II, Random III and Regular. The crop density varied from 60 to 540 plants/m². In the case of the random patterns, the degree of randomness referred to the non-uniform spatial distance from a reference point, as generated by random numbers. In the regular pattern, there was a uniform plant to plant spacing in a grid pattern. In all these four spatial treatments, three densities were established, namely dense - 27 seeds/pot; medium - 9 seeds/pot and sparse - 3 seeds/pot.

In all the experiments, the spatial arrangements were obtained by scattering an equal number of seeds at pre-determined locations within the surface soil area. Proper crop production measures were undertaken during the crop growing season. Growth chamber temperatures were programmed to maintain day and night temperatures of 30°C and 25°C, respectively. After attaining maturity, the plants were harvested and the yield components were determined. Data on grain yield were statistically analysed by the t-test using the Excel package.

Results and discussion

Extent of non-uniform crop establishment by row seeding method in Field A

Figure 1 shows the magnitude of difference in grain yield of 225 plants and their intra-row spacing along an 800 cm track. The average yield was calculated to be 6.34 g/plant; the range and standard deviation values were estimated to be 6.90 and 1.82 g/plant, respectively. The coefficient of variation was 29%. This variation must have been caused by the sub-optimal intra-row spacing, because with a single cultivar over a small distance the difference in yield cannot be attributed to any other environmental cause. The linear relationship established by correlating the spacing between two consecutive plants and their average grain yield was given by the equation $y = 5.67 + 0.19 S$, where y is the average grain yield (g/plant) and S is the intra-row spacing (cm). This investigation and other studies (Anbumozhi, 1995) indicate the importance of establishing optimal intra-row spacing when direct seeding methods are employed. It can be concluded that the area available to each plant has a significant influence on the final grain yield.

Magnitude of non-uniform crop establishment by broadcasting method in field B

The number of plants counted in a 50×50 cm cell area and the grain yield at 18 locations within Field B showed non-uniform crop stands throughout the field. The lowest plant count was 9 plants in a 0.25 m^2 cell, thus the area available to each plant was 277 cm^2 , whereas the highest plant count was 25 plants in a 50×50 cm cell, reducing the area available for each plant to 100 cm^2 . This site-specific non-uniformity and localized competition affected the average grain yield and gave rise to uneven crop production within the same field.

This density effect is given by the relationship depicted in Figure 2:

$$y = 34 - 1.5 P \quad (r = -0.83).$$

If Y is the total grain yield in a particular cell,

$$\begin{aligned} Y &= y \times P \\ &= (34 - 1.5P)P \\ &= -1.5 P(P - 23) \end{aligned}$$

where y is the average grain yield (g/plant), Y is the total grain yield (g/ 0.25 m^2) and P is the number of plants in 0.25 m^2 . From this, the optimum value can be calculated as $P_{opt} = 11.5$ plants/ 0.25 m^2 or 46 plants/ m^2 . This optimum plant density of 46 plants/ m^2 corresponds to 0.022 m^2 /plant implying a precise plant to plant spacing of 15 cm, if a regular square grid pattern is to be established in direct seeding methods. These relationships show a decrease in yield under sub-optimal crop densities. If the total number of plants in a particular area is less than the optimum seed rate, then crop production is also less than the optimum. Further, it can be concluded that competition is not merely a communal effect reducing the yields of all plants equally; there may be detectable localized

variations in the competition from neighbouring plants, i.e. plant-plant spacing. Hence, establishment of uniform, optimum crop stands with the appropriate seed rate is an important consideration in broadcasting methods.

Effect of changes in intra-row spacing on rice crop yields

Table 1 depicts the influence of changes in intra-row spacing on rice crop production as the plant density varied from 50 to 1000 plants/m². In the 1994 study, the yield increased with an increase in spacing until 2 cm, after which it started declining as the number of plants decreased. The results of the 1995 study also confirmed the existence of an optimum intra-row spacing. Again that optimum spacing occurred at 2 cm, with a crop density of 100 plants/m², producing the maximum grain yield of 7.26 t/ha, which is also consistent with the shoot and root yields. The narrowest intra-row spacing, 0.5 cm, yielded only 6.07 t/ha, resulting in a total yield loss of 16.4% compared with optimum. In the same way, the widest spacing, 5 cm, produced 37.1% less than with an intra-row spacing of 2 cm. Naturally, in both years with an increase in spacing the average grain yield increased. Figure 3 depicts the linear relationship obtained between intra-row spacing and grain yield. The equation so obtained was:

$$y = 0.70 S + 0.58 \quad (r = 0.96)$$

where, y = average grain yield (g/plant); S = intra-row spacing (cm).

This increase in yield with increased row spacing is attributable to the increased area available to each plant. With equal amounts of nutrients available in the specified area, less densely spaced plants had better root growth, contributing more yield. Nevertheless, wider spacing carries with it the problem of the under-utilization of nutrient resources, due to the less optimized surface available to individual plants. Moreover, the supply — demand balance is also affected, which is understandable seeing the number of plants lost during the growth process under different spacings. As can be seen in Table 1, mortality rates of 18.8%, 13.7%, 15.4% and 11.8% were calculated at 0.5 cm, 1.0 cm, 2.0 cm and 3.0 cm spacings, respectively. Even though there was little or no plant loss at wider spacings, the total grain yield was much less than at 2 cm intra-row spacing.

Effect of precise geometrical arrangement on rice crop yields

Table 2 shows the effect of plant-plant spacing on crop yield. Maximum yield (grain and dry matter) was observed at 8 × 8 cm spacing, giving a crop density of 69 plant/m², on a regular grid pattern. A too narrow spacing of 4 × 4 cm (crop density 625 plants/m²) produced 21.5% less grain yield compared to moderate spacing of 8 × 8 cm where a maximum grain yield of 7.84 t/ha was achieved. The yield reduction caused by a too wide spacing of 12 × 12 cm (crop density 156 plants/m²) was 28.8% compared to the optimum spacing. Naturally this could be related to the under-utilization of available resources. The experimental results indicate that the better the available space is exploited, the greater the crop yields.

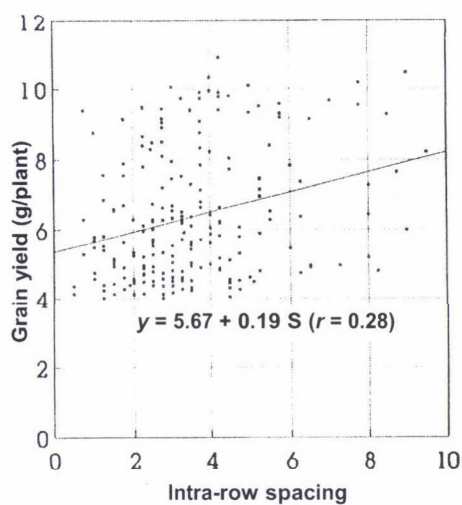


Fig. 1. Relationship between intra-row spacing and grain yield in Field A

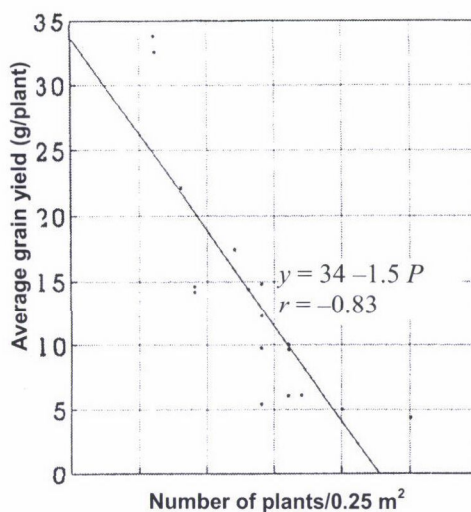


Fig. 2. Relationship between crop density and grain yield in Field B

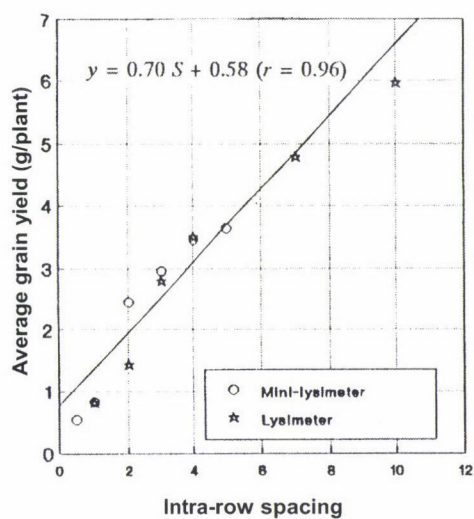


Fig. 3. Relationship between intra-row spacing and grain yield in lysimeters

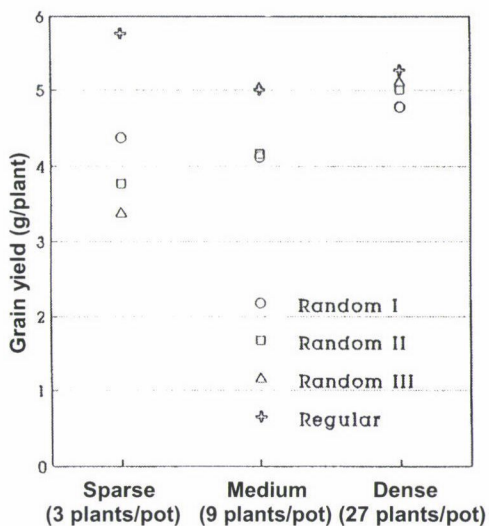


Fig. 4. Effect of changes in random and regular spatial arrangement on grain yield

Table 1
Effect of intra-row spacing on paddy yield in lysimeter experiments

Intra-row spacing (cm)	Grain yield (t/ha)	Shoot yield (t/ha)	Root yield (t/ha)	No. of plants at seeding	No. of plants at harvest	Mortality rate (%)
1994						
1	3.29 ^a	5.06 ^a	0.27 ^b	51	NC	—
2	2.92 ^{ab}	5.45 ^a	0.16 ^b	26	NC	—
3	3.71 ^a	5.22 ^a	0.21 ^b	17	NC	—
4	3.66 ^{ab}	5.07 ^a	0.13 ^a	13	NC	—
7	2.87 ^b	4.08 ^b	0.17 ^a	7	NC	—
10	2.68 ^a	2.39 ^a	0.13 ^a	5	NC	—
1995						
0.5	6.07 ^c	7.80 ^b	0.88 ^b	101	82	18.8
1	4.79 ^c	5.78 ^b	0.58 ^{ab}	51	44	13.7
2	7.06 ^c	8.49 ^{ab}	0.97 ^{ab}	26	22	15.4
3	5.70 ^b	6.40 ^{ab}	0.62 ^{ab}	17	15	11.8
4	5.13 ^b	6.08 ^a	0.70 ^a	13	13	0
5	4.57 ^a	4.85 ^a	0.51 ^a	11	11	0

In each column, means followed by a common letter are not significant at the 5% level; NC – Not counted

Table 2
Effect of plant-plant spacing in mini-lysimeter experiments

Plant to plant spacing (cm × cm)	Yield (t/ha)			No. of plants at seeding	No. of plants at harvest	Mortality rate (%)
	Grain	Shoot	Root			
4 × 4	6.48 ^a	8.24 ^b	1.12 ^a	96	73	23.9
8 × 8	7.84 ^b	12.59 ^a	0.55 ^{ab}	24	24	0
12 × 12	5.06 ^a	9.21 ^a	1.16 ^a	8	8	0

In each column, means followed by a common letter are not significant at the 5% level

Effect of random spatial arrangement on rice crop yields

As a result of broadcasting the seed at random, the area ascribable to each plant varied and affected the crop yield. The crop density also affected plant growth and finally grain yield. The maximum grain yield of 5.76 t/ha was obtained in the sparse treatment with a regular spatial arrangement. As shown in Figure 4, in the medium and sparse treatments the difference in yield between plants within the treatment was more due to the improper utilization of the space. The results of all the density treatments showed that as the arrangement tended towards a regular grid type, more grain was produced. This could have been the result of either reducing the interference between plants, expressed as active interference, or of reducing the resource space available to individual plants, referred to as passive interference. However, interference, most likely passive, between the plants would have been greater in the dense treatment where there was no effect of the spatial arrangement on total grain yields. The

better utilization of the space in the regular square arrangement probably arose for two reasons. First, allowing that plants usually show some form of radial growth, it would appear that plants in a square arrangement were able to grow for a longer period before encountering the space occupied by the neighbours; secondly, the distance plants had to grow to exploit all the available space was less in a random arrangement than in the case of regular arrangement; hence all the available space was exploited earlier. The results of this study lead to the general principle that the more uniform the exploitation of the available space, the greater the uniformity of crop yields.

Conclusion

Field studies demonstrated that the distance away from neighbours had an effect on crop yields because there were detectable localized variations in the competition from neighbouring plants. Experiments in lysimeters on intra-row spacing showed the existence of an optimum intra-row spacing of 2 cm with a crop density of 100 plants/m², which could be applied in row seeding methods. The results of mini-lysimeter experiments emphasized the importance of keeping a moderate spacing of 8 × 8 cm or a plant population of 156 plants/m² if a precise geometric square grid plant arrangement is to be adopted in direct seeding methods. Pot experiments showed that the crop yield can be increased markedly with increased plant density and a significant yield advantage can be obtained from regular planting over random seeding. Further, random patterns cause a range of micro-scale variations in grain yield. Through these studies it was made clear that the manipulation of spatial geometry has promising potential for increased crop yield. Labour can be saved and yield gains can be achieved by mechanized direct seeding that adopts the optimum spatial arrangements described above.

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IMPORTANCE OF LOCAL SOIL ERODIBILITY MEASUREMENTS IN SOIL LOSS PREDICTION

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Soil is a non-renewable natural resource. An accurate estimate of soil loss is essential for making agricultural policy decisions and for planning land use at all scales from farm to national levels. A wide range of erosion models, most of them combined with GIS and varying in purpose and details, are available for predicting soil loss. The Universal Soil Loss Equation (USLE) consists of statistical summaries of annual average soil loss data from plot studies in the United States. The equation has been worked out on the basis of the data of over 10,000 plot-years collected on erosion plots in the USA. However, the basic problem is the possibility of transferring empirical relations from the climatic and soil conditions in which they are measured into other geographical areas. This paper investigates the possibility of calculating the soil erodibility factor of the USLE with rainfall simulations and its importance in soil loss prediction.

Key words: erodibility, prediction, soil erosion, erosion modelling

Introduction

It started in 1915. A young man went to a professor with a small problem. The young man was instructed to measure rainfall and runoff over a two-month period. After the first runoff, the mud was weighed and dried. The measurements revealed a loss of soil carrying plant nutrients in excess of that removed by the crop (Woodruff, 1987).

In 1929 Hugh Hammond Bennett successfully campaigned for funding from the U.S. Congress to begin soil erosion research in the United States. He initiated the collection of data, which Wischmeier later compiled into one large database, referred to as the USLE Database. The USLE database is a collection of files containing over 11,000 plot-years of data from 47 locations in 24 states. This data was collected in the 1930s, 1940s and 1950s, then put into the computer using punch cards during the 1950s.

Soil erosion has been examined with rainfall simulation from various aspects. Lang et al. (1984), Harmon and Meyer (1978), Lattanzi (1973) and Kerényi (1981) examined splash erosion under artificial rainfall on small erosion plots. Inter-rill erosion required wider and longer plots (Neal, 1938; Zingg, 1940; Lattanzi, 1973; Andrews, 1981). Van Liew and Saxton (1983), Meyer and Harmon (1989) and Quansah (1985) carried out research on rill erosion. Rainfall simulators were used to described larger areas by Gilley et al. (1977), Hahn et al. (1985), Hart (1984), Mitchell et al. (1983) and Kertész et al. (1997). Finally, rainfall simulators were investigated by Auerswald és Eicher (1992) and Auerswald et al. (1992a, b).

Lang et al. (1984), Kertész et al. (1997) measured soil erodibility under artificial rainfall. Stein and Nett (1997) examined the erodibility of reclaimed surface mined areas. Loch et al. (1998) carried out soil erodibility measurements on Australian soils.

USLE can be used all over the world, but only after local calibrations. In Hungary calibrations were made for calculating the K factor based on measurements under natural rainfall (Kertész et al., 1997). However, these results were not published in Hungarian and the soil types were not classified on the Hungarian Soil Classification System.

To obtain reliable K values, several requirements must be fulfilled. Measuring K values under natural rainfall means collecting a large database of annual soil losses obtained over a period sufficiently long to reduce variations in antecedent soil water, surface conditions and other hydrological variations (Torri et al., 1997). This period is assumed to be 20–22 years (Wischmeier, 1976) in the continental US but may differ in other parts of the world (Römkens, 1985). This is why scientists use rainfall simulators for collecting data. It is faster and easier.

The purposes of this paper were:

- to measure and compare the K factors of different soil types,
- to compare K factor estimations with measurements,
- to highlight the effects of estimations versus measurements in soil loss estimations.

Materials and methods

Estimating soil loss with the USLE model

The standard reference for erosion modelling is the Universal Soil Loss Equation (USLE) (Wischmeier and Smith, 1978). New models have been developed since 1978 but their input requirements are too high and the database is not available. This is the reason why USLE was chosen for the present work.

The USLE is an empirical model that uses physical factors to quantify the amount of soil lost per hectare per year. Its well-known equation is: $A = R * K * L * S * C * P$

where A = Soil Loss ($t * ha^{-1} * y^{-1}$),

R = Rainfall Erosion Index ($MJ * mm * ha^{-1} * h^{-1} * y^{-1}$),

K = Soil Erodibility Factor ($t * ha * h * ha^{-1} * MJ^{-1} * mm^{-1}$),

L = Slope Length (dimensionless),

S = Slope Gradient Factor (dimensionless),

C = Cropping Cover Management Factor (dimensionless),

P = Agricultural Practice Factor (dimensionless).

Procedure for using USLE

1. Determine the R Factor.
2. Determine the K value, based on the nomograms of Wischmeier and Smith (1978), on an equation based on soil characteristics or on measurements under artificial rain.
3. Divide the field into sections of uniform slope gradient and length. Assign an LS value to each section.
4. Choose the crop type factor for the crop to be grown.
5. Select the P factor based on the tillage practice to be used.
6. Multiply the 5 factors together to obtain the soil loss per acre.

Database for USLE

A scale of 1:10 000 was used. The databases for the R and P factors were not available on this scale. These were constant.

The methods of Hickey et al. (1994) and Desmet and Govers (1996) were used for calculating the LS factors with GIS Arc/Info. Pataki (2000) prepared an erosion map on a scale of 1:10,000 in Hungary.

Technical support

ESRI (Environmental Systems Research Institute, Inc.), NT Arc/Info 7.2 was used with a Unix operation system for digital work and calculations and ESRI Arc/View 3.1 with a Win98 operation system for presentation.

Results and discussion*Soil erodibility (K factor) measurements*

The second factor of USLE is the K factor. Rainfall simulations were made in the Balaton watershed to give a better calibration of K factors in the region. The K factors varied from 0.008 to 0.04 ($t * ha * h * ha^{-1} * MJ^{-1} * mm^{-1}$). Table 1 shows the results of calculations made for the two soil types examined in Nemessándorháza.

Two-way interaction analysis of variance showed significant differences between the K factors of the soil types. It can also be stated that the soil types form different groups under various rainfall intensities. So far erosion prediction with USLE has worked with one K factor for a whole year. The statistical analysis of rainfall simulation studies showed that K factors not only vary by soil types, but the K factor of each soil type varies according to soil moisture content. Since soil moisture content mostly varies by season, the seasonal variability of K factors can be investigated in rainfall simulation studies. It is clear that for erosion modelling more than one K factor is necessary during the year for proper calculations.

Table 1
Results of K factor calculations from rain simulation data

611	K _{dry}	0.004272	612	K _{dry}	0.001310	Mean of	K _{dry}	0.002791
	K _{damp}	0.009688		K _{damp}	0.010985	611+612	K _{damp}	0.010336
	K _{wet}	0.009620		K _{wet}	0.013165		K _{wet}	0.011392
	K _{damp+wet}	0.009633		K _{damp+wet}	0.012750		K _{damp+wet}	0.011191
	K _{total}	0.008740		K _{total}	0.010919		K _{total}	0.009830
613	K _{dry}	0.001573	614	K _{dry}	0.003490	Mean of	K _{dry}	0.002532
	K _{damp}	0.009867		K _{damp}	0.012201	613+614	K _{damp}	0.011034
	K _{wet}	0.011070		K _{wet}	0.010267		K _{wet}	0.010669
	K _{damp+wet}	0.032522		K _{damp+wet}	0.010654		K _{damp+wet}	0.021588
	K _{total}	0.009682		K _{total}	0.009460		K _{total}	0.009571

Comparison of calculated and estimated K factors

The conversion of the imperial dimensions of the K factor to SI means multiplying it by 0.13. This means that in the SI system the K factor may be as high as 0.1 ($t * ha * h * /ha^{-1} * MJ^{-1} * mm^{-1}$) (Foster et al., 1981). Former estimations were made before the overview of problems in translating different units. Estimations for soil types were made by Stefanovits (1966) and can be seen in Table 2. Stefanovits estimated K factors between 0.25 and 0.6 and dimensionless, while soil erodibility calculations through rain simulation studies showed highest values of 0.04, so estimations resulted in 150 times the calculated values.

Preparation of basic maps for the preparation of a soil loss estimation map

R factor map: since there is no detailed map for the R factor on this scale, the R factor was constant ($= 415 MJ * mm * ha^{-1} * h^{-1} * y^{-1}$).

K factor map: rain simulation data were used to obtain the K factor map of Nemessándorháza. Two soil erosion prediction maps were prepared, one using estimated and one using calculated K factors (Table 3).

USLE is not able to calculate sedimentation, so wherever the properties of the soil types suggested this (e.g. Cambisols, Fluvisols) a zero K factor was given to show these soil types as potential sedimentation areas (Fig. 1).

L and S factors: the Digital Elevation Model was the base for the L and S factor map. The map was prepared as described in the Materials and methods section. L and S factors play a very important role in erosion modelling.

C factor map: A 1:10 000 scale Unified Projection System map was the base for the C factor map. The USLE manual (Wischmeier and Smith, 1978) was used for the C factor classification of land cover and vegetation.

P factor map: The P factor was constant ($=1$), since upslope-downslope cultivation was widespread.

Table 2
K factor estimation for soil types (Stefanovits, 1966)

Texture	Highly eroded soils	Chernozems	Brown forest soil (BFS)	BFS with clay accumulation in B horizon
Sand	0.45–0.55	0.35–0.45	0.35–0.45	0.40–0.50
Sandy loam	0.50–0.60	0.35–0.45	0.30–0.40	0.30–0.40
Loam	0.50–0.60	0.30–0.40	0.25–0.35	0.25–0.35
Clayey loam	0.45–0.53	0.25–0.35	0.25–0.35	0.25–0.35
Clay	0.40–0.50	0.25–0.35	0.25–0.35	0.30–0.35

Table 3
Comparison of estimated and calculated K factors for Nemessándorháza

Estimated K (Stefanovits, 1981)	Calculated K (rain simulation studies)
0.0001	0.0001
0.0010	0.001
0.3	0.00862
0.3	0.00891
0.3	0.00894
0.32	0.018
0.55	0.03838

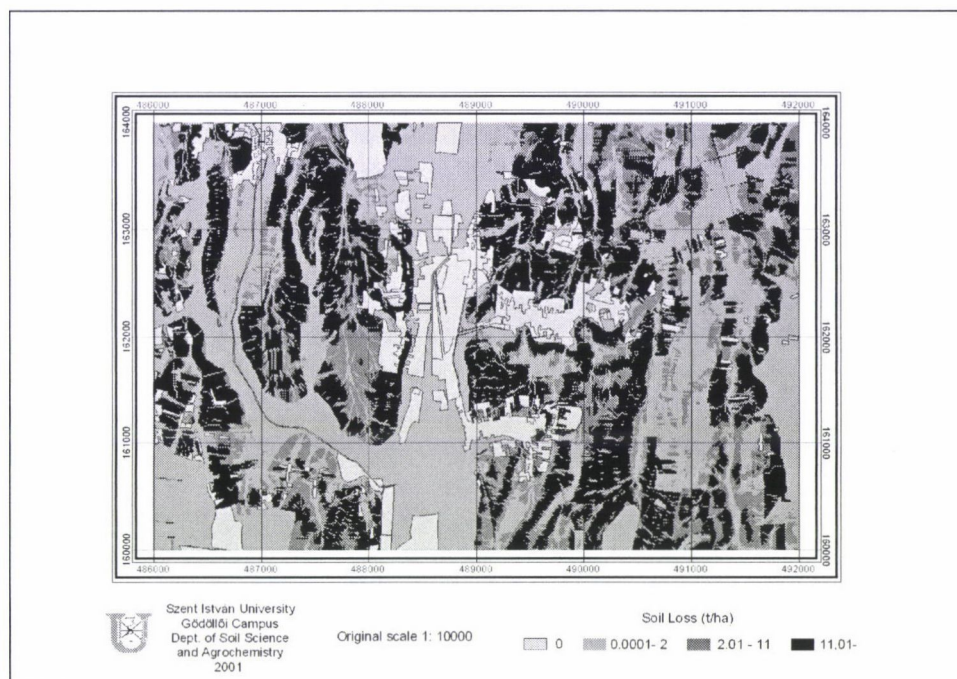


Fig. 1. Soil loss estimations with estimated K factors for Nemessándorháza

Explanations of colours and soil loss categories

Digital maps were prepared of all factors that were needed for soil loss estimations with the USLE model. The Arc/View shape files had to be converted to grid files. When all the maps of USLE factors were converted to grid, the values were multiplied. The result is the soil loss estimation and can be seen on Figures 1 and 2. The map in Figure 1 was prepared using estimated K factors and the map in Figure 2 using calculated K factors.

The white areas are those where there is no threat of erosion or there are areas of water or settlements.

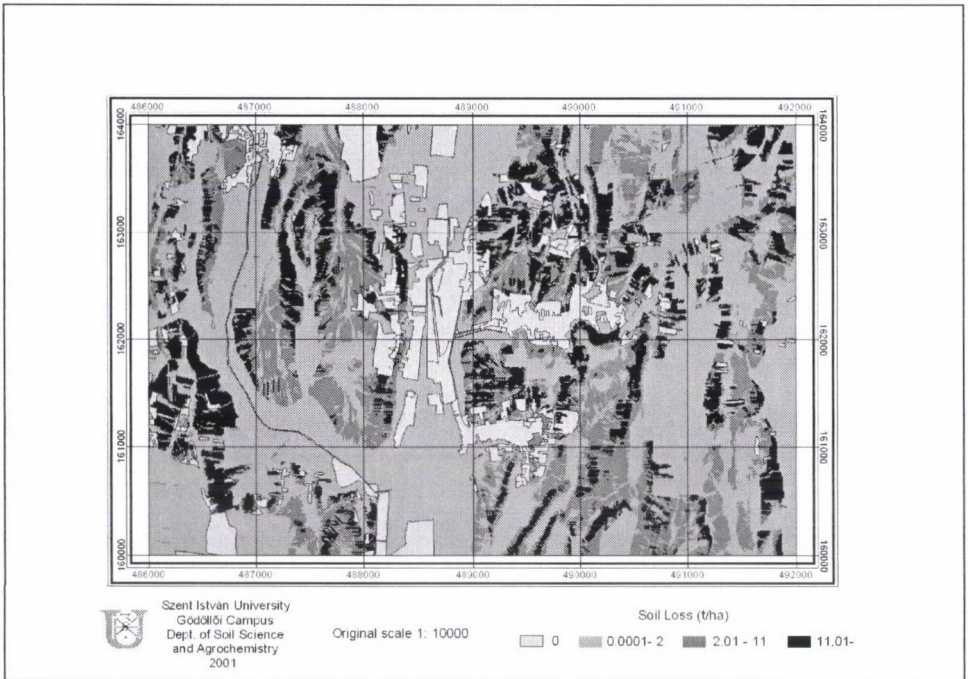


Fig. 2. Soil loss estimations with calculated K factors for Nemessándorháza

Sustainable areas are in light grey. This category is $0\text{--}2 \text{ t} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$. The upper limit of this class reflects the average rate of soil formation on intensive agricultural land.

The maximum allowable soil loss is $11 \text{ t} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$. This is the maximum rate of soil formation under optimal conditions. The USLE manual does not require farmers to protect their lands against erosion up to the limit of $11 \text{ t} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$. If the average soil formation rate is about $2 \text{ t/ha} \cdot \text{year}$ than there is a real chance of soil degradation.

Where the maps show black colour, the predicted soil loss is above the acceptable limits even for USLE. Nevertheless, farming is still carried out and farmers use unsuitable methods of soil and crop management. In the USA efforts are being made to reduce soil erosion to sustainable limits.

Comparison of prediction with estimated and measured K factors in Nemessándorháza

The total area of the map is 24 km^2 . Settlements, roads and potentially eroded areas cover about 22% of the total area. Calculations for the comparison of erosion on different scales were made on eroded areas (Table 4).

The comparisons reflect the results of figures in estimated and calculated K factors. Prediction with estimated K factors overestimates the highly eroded areas (above $11 \text{ t} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$) and underestimates areas of erosion in the low category.

This means that soil loss prediction with estimated K values results in an overestimation of erosion hazards and the need for soil protection measures.

Table 4

Comparison of eroded land distribution prepared using estimated and calculated K factors for Nemessándorháza

Prediction with estimated K factor		Prediction with calculated K factor	
Categories	%	Categories	%
0–2 t * ha ⁻¹ * y	41.118	0–2 t * ha ⁻¹ * y	54.361
2–11 t * ha ⁻¹ * y	22.221	2–11 t * ha ⁻¹ * y	28.629
11– t * ha ⁻¹ * y	36.66	11– t * ha ⁻¹ * y	17.009
Total:	100	Total:	100

Conclusions

The data presented in this paper give an initial database for brown forest soils in the Western Balaton Watershed. The data can be used for similar soils and provide a basis for estimations where local data are not available or too expensive to measure. However, for exact estimations local measurements are strongly recommended.

More than one K factor is needed per soil type to give proper estimations of soil loss. As rainfall simulation experiences proved, K factors varied with the intensity of precipitation and the initial moisture content of the soil besides other soil characteristics that were used for establishing the USLE equation.

Two-way variance analysis proved K factors, soil types and rainfall intensities to have combined effects. Rainfall intensities resulted in different K factors and soil types resulted in different K factors and the factor effects were vice versa.

Stricter regulations are needed for soil conservation measures. It is not acceptable to allow agricultural production where the soil loss rate exceeds the soil formation rate. These areas can be offered for nature conservation, forestation or pasture. Land use changes must be decided on.

Baseline data from virgin soils are lacking in most cases for establishing soil loss tolerance values. For proper soil loss rating, soil formation must be at least estimated. Otherwise some other method should be established for representing soil loss tolerance values.

The USLE is a comprehensive technique available for field use in estimating cropland erosion. More emphasis must be laid on the dimensions of the USLE factors. Researchers must be aware of differences between US Imperial, SI and metric systems. A common system is needed for further international cooperation.

The USLE is capable of providing a convenient working tool for conservationists. More *in situ* field measurements are needed to outline soil characteristics. Such data are also needed for more advanced, process based models. So far no data are available for these models and as long this is so, it means more errors using models with estimations of approximately 50–100 factors than using the USLE model.

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RESPONSE OF SORGHUM TO NITROGEN AND PHOSPHORUS FERTILIZATION IN SEMI-ARID ENVIRONMENTS IN WELO, ETHIOPIA

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Information on the response of sorghum to N and P fertilizers in the semi-arid areas of Welo is scanty. Field experiments were conducted at three semi-arid locations, for two years at each location, to investigate the response of sorghum to N and P fertilization. Four levels of each of N (0, 23, 46 and 69 kg ha⁻¹) and P (0, 10, 20 and 30 kg ha⁻¹) were studied in factorial combinations in a randomized complete block design (RCBD) with triplicates. The results revealed substantial responses of sorghum to N fertilization for almost all parameters, whereas the frequencies of significance for P and for N and P interactions were very low. The sorghum grain yield, stover yield, total aboveground biomass and panicle weight increased significantly ($P < 0.01$ and 0.05), some in linear and others in quadratic responses to N fertilization at all locations. These represented increases of 17–35%, 13–27%, 16–27% and 16–32%, respectively, with significant N treatments. Thousand-kernel weight was responsive to N fertilization only at one of the three locations. Responses to P fertilization were variable between seasons and locations due possibly to the high initial soil P. Grain yield and thousand-kernel weight increased in linear and quadratic responses to P fertilization only at Mersa and Chefa. Panicle weight responded to P application only at Chefa. Total biomass weight was significantly increased in response to P application only at Mersa. Stover yield did not respond to P fertilization.

Key words: semi-arid areas, fertilizer economics, moisture stress, *Sorghum bicolor* (L.) Moench

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the major grain crop of the semi-arid areas of northeastern Ethiopia. Sorghum plays an appreciable role in supplying the population of this part of the country with protein, carbohydrates and minerals. Moreover, sorghum stalk is a dependable source of firewood, feed and construction material (Hailemichael, 1998). However, the average mean grain yield of sorghum in this part of the country is low (approx. 1.2 t ha⁻¹) and variable, mainly due to moisture deficiency and nutrient stress. The soils in these areas are very poor in soil nutrients owing to intensive soil erosion, continuous cereal growing and a long cropping history (Hailu and Kidane, 1988; Kidane and Getachew, 1994). Despite the poor status of soil nutrients, farmers in North-eastern Ethiopia apply neither mineral nor organic fertilizers to their sorghum fields. This situation has led to the progressive depletion of soil nutrients. In a cropping system where large quantities of nutrients are exhausted by erosion and exported in harvested products, it is unlikely that sustainable high yields will be obtained without nutrient replenishment.

Despite the poor soil fertility status in the semi-arid areas, poor response to fertilizer application has been reported mainly because of moisture stress (Asnakew, 1994; Probert et al., 1994). Nevertheless, findings of many studies in semi-arid areas of other countries showed increased grain production with the application of even high rates of fertilizers (Myers, 1978; Hibberd et al., 1991; Reddy and Kidane, 1993). Doyle and Holford (1993) also reported profitable wheat yield and protein responses to very high rates of N fertilizer in spite of the relatively dry conditions in Australia. They have also found the agronomic efficiency of fertilizer N to be comparable with that of moister regions of the world.

The problem of declining soil fertility under cropping is most alarming in the semi-arid regions (Probert et al., 1994). Most soils in the semi-arid areas of northeastern Ethiopia tend to exhibit low total N, available P and organic C (Asnakew, 1994; Kidane and Getachew, 1994). Therefore, the application of mineral fertilizers is essential to sustain crop production and feed the growing population in these regions. However, information on the response of sorghum to N and P fertilization in these areas is scanty. What is currently under use is a blanket recommendation of 41 kg N ha⁻¹ and 20 kg P ha⁻¹ developed by extrapolating from studies made in other similar environments in the country. It has also been observed that the blanket recommendation has failed to increase sorghum yield and questions have been raised from users. It has often been indicated that the blanket recommendation could be either below or above the optimal rate, which in either case could cause the farmer economic loss. Thus, the main objective of this study was to investigate the response of sorghum to different levels of N and P fertilizers under semi-arid environments in Welo.

Materials and methods

Sites

These experiments were conducted at the three research sites of the Sirinka Agricultural Research Center, namely: Mersa (in 1997 and 1998), Chefa (in 1998 and 1999) and Kobo (in 1998 and 1999). The test sites are located at 11° 42' N and 39° 36' E (Mersa), 10° 57' N and 39° 47' E (Chefa) and 12° 9' and 39° 38' E (Kobo). The mean elevation of each location is 1580 m, 1450 m and 1470 m above sea level, respectively. The growing season at the experimental sites is characterized by high temperature, high evaporative demand, reduced available moisture periods, and unevenly distributed rainfall. The mean monthly evapotranspiration exceeds rainfall except during July and August (data not shown). In these locations, moisture shortage often occurs during the two most critical periods of sorghum growth (anthesis and grain formation).

Experimental design

In all years and sites, the experiments were set up in a randomized complete block design with three replications where the factorial combination of four levels each of N and P constituted the treatments. Nitrogen, as urea (46-0-0 N-P-K), was applied at rates of 0, 23, 46 and 69 kg ha⁻¹ and P as triple superphosphate (0-46-0 N-P-K) was applied at 0, 10, 20 and 30 kg ha⁻¹.

Experimental procedure

Half the N and all the P were applied in a band preplanting and incorporated into the soil. The remainder of the N was side dressed at approximately the six to eight leaf stage of the crop. Sorghum cultivars 76 T1 #23 at Kobo and Gambella 1107 at Mersa and Chefa were hand drilled in a row and thinned to an interplant spacing of 15 cm 15 days after 50% emergence to obtain a plant population of 89,000 plants ha⁻¹. Plot size was seven rows 75 cm wide and six metres long. At all sites plantings were made in tied ridges. Planting at each site occurred in early July each year. Cultural practices for weed and insect control were conducted on an as-needed basis at all sites. Prior to planting, composite surface (0–20 cm) and subsurface (20–40 cm) soil samples from five points across the study area were collected and analysed for soil physicochemical properties following the procedures outlined by Page et al. (1982). The innermost rows of each plot were hand harvested for grain and biomass yield at maturity. The panicles were weighed air-dry and hand threshed. Grain moisture content was determined by electrical resistance and yields were adjusted to 12.5% moisture. The biomass from the areas where panicles were harvested was removed at ground level and weighed to obtain air-dried total biomass weight (TBW). Sub-samples of the stover were taken, weighed and dried at 70°C to constant weight to determine the stover dry matter yield. Thousand-kernel weight (TKW) was determined by weighing 1000 seeds and the values were adjusted to 12.5% moisture. The plant population per plot was below the desired population (88,888 plants ha⁻¹) due to uneven germination resulting from moisture stress at germination. However, no attempt was made to adjust the plant population per plot, as the difference between the different plots was not statistically significant.

Analysis of variance for the measured parameters was performed using the MSTATC statistical computer program (MSTATC, 1989) for each individual year and combined data. Combined analyses were performed, as the data were found to be homogeneous for all parameters. Grain yield is reported for both the individual years and the combined data, but for the other parameters only the results of the combined data are reported. Combined analysis over sites was not done as the three sites represent different agro-ecologies. Differences between treatment means were delineated using the Least Significant Difference test ($P < 0.05$).

Soil physicochemical properties and rainfall conditions

The physicochemical properties of the soils of the experimental sites are summarized in Table 1. At all locations the soils had poor total N (%) and high available P (Olsen, ppm). During the experimental years, the rainfall received was above the long-term average by 49–58% at Mersa, 38–64% at Chefa and 56–58% at Kobo (Fig. 1). The high amount of total seasonal rainfall is misleading, as great variability often exists in its distribution. The rainfall distribution in the experimental years was poor, since much of the seasonal rainfall (46–86% at Mersa, 71–91% at Chefa and 76–91% at Kobo) occurred only in July and August (Fig. 1). The amount of rainfall and number of rainy days from mid-August onwards and in September, when the crop requirement is at a maximum, was too poor for successful grain formation. Due to this rainfall pattern, these experimental sites are often characterized as semi-arid. The total number of rainy days and the number of rainy days with above 10 mm rainfall declined from mid-August onwards at all sites, which indicates the occurrence of moisture stress during the flowering and grain formation stages of the crop (Fig. 1).

Table 1
Physicochemical properties of the soils in the experimental sites

Properties	Kobo			
	1998		1999	
	0–20 cm	20–40 cm	0–20 cm	20–40 cm
pH (1:1 H ₂ O)	7.2	7.3	7.4	7.6
Total N (%)	0.10	0.09	0.10	0.09
Available P (Olsen, ppm)	18.48	17.04	20.1	14.3
Organic C (%)	1.337	1.315	1.317	1.339
Ca ²⁺ (cmol kg ⁻¹)	22.02	24.70	20.96	21.24
Mg ²⁺ (cmol kg ⁻¹)	7.04	6.43	17.81	16.34
Na ⁺ (cmol kg ⁻¹)	0.27	0.32	0.27	0.29
K ⁺ (cmol kg ⁻¹)	0.45	0.30	0.44	0.40
CEC (cmol kg ⁻¹)	51.71	52.89	42.88	33.94
Clay (%)	44.75	43.25	44.75	43.25
Silt (%)	33.0	32.5	33.0	32.5
Sand (%)	22.25	24.25	22.25	24.25
Textural class	Clay	Clay	Clay	Clay
Chefa				
pH (1:1 H ₂ O)	6.5	6.6	6.4	6.4
Total N (%)	0.22	0.18	0.19	0.16
Available P (Olsen, ppm)	58.75	70.33	30.48	23.28
Organic C (%)	1.891	1.978	2.052	1.830
Ca ²⁺ (cmol kg ⁻¹)	26.84	25.63	24.75	25.49
Mg ²⁺ (cmol kg ⁻¹)	13.43	11.28	6.87	6.60
Na ⁺ (cmol kg ⁻¹)	0.25	0.24	0.40	0.40
K ⁺ (cmol kg ⁻¹)	0.88	0.76	0.63	0.44
CEC (cmol kg ⁻¹)	47.97	38.71	61.88	58.76
Clay (%)	59	60.63	64.75	63.25
Silt (%)	20	21.25	20.5	25.0
Sand (%)	21	18.13	14.75	13.75
Textural class	Clay	Clay	Clay	Clay
Mersa				
pH (1:1 H ₂ O)	7.1	7.1		
Total N (%)	0.25	0.18		
Available P (Olsen, ppm)	42.1	55.8		
Organic C (%)	1.48	1.42		
Ca ²⁺ (cmol kg ⁻¹)	17.7	18.06		
Mg ²⁺ (cmol kg ⁻¹)	8.69	8.03		
Na ⁺ (cmol kg ⁻¹)	0.30	0.27		
K ⁺ (cmol kg ⁻¹)	0.39	0.41		
CEC (cmol kg ⁻¹)	45.43	44.19		
Clay (%)	57.25	57.25		
Silt (%)	22.5	23.0		
Sand (%)	20.25	19.75		
Textural class	Clay	Clay		

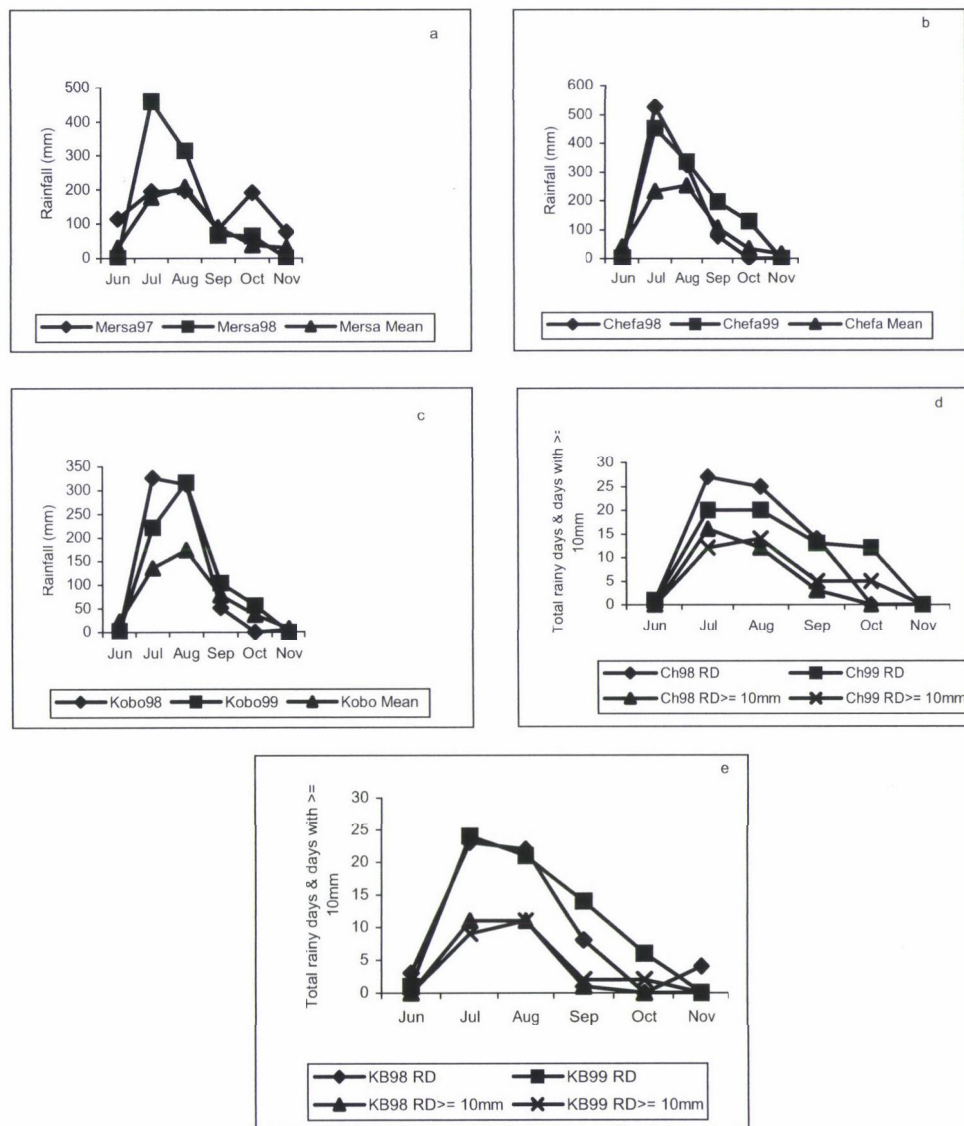


Fig 1. Experimental years and long-term growing season rainfall data: a) Mersa, b) Chefa, c) Kobo, d & e) total number of rainy days and rainy days with ≥ 10 mm rainfall at Chefa and Kobo, respectively. Long-year averages of each site are: Mersa = 29 years average (1962–1995); Chefa = 35 years average (1954–1988); and Kobo = 13 years average (1973–1996). Mersa and Kobo have some years missing in the indicated ranges

Results and discussion

General treatment effects

Considering the five crop parameters assessed in each of the six site-years and the three combined data, the effects of each experimental factor and the interactions between them were measured in a total of 135 comparisons, out of which significant effects were recorded in 48 comparisons. Of these 48 comparisons, significant differential effects on crop parameters were predominantly due to N rates (34 of 48), indicating that N is the most important nutrient element limiting sorghum growth and yield. The frequencies of significance of P rates (11 of 48) and N and P interactions (3 of 48) were very low.

Field observations revealed that N-treated plants tended to have dark green, larger leaves, thick stalks and dense canopy until booting. Phosphorus-treated plants also tended to be taller with larger leaves and dense canopy than non-treated plants. However, both effects were less noticeable after sorghum heading. The less perceptible physical difference from heading onwards was possibly due to moisture stress occurring from September onwards.

There were also large significant ($P < 0.01$) differences between years in all parameters at Chefa, in four parameters at Mersa and in three parameters at Kobo (Table 2). For example, grain yield in the second year (1999) was significantly ($P < 0.01$) greater by 29% at Chefa and 10% at Kobo. The higher grain yields in the latter year reflected better total seasonal rainfall in general and during grain formation (in September) in particular (Fig. 1).

The grain yield at Chefa was relatively higher, which could probably be attributed to the fact that the environmental conditions were more suitable for the sorghum variety, Gambella 1107.

Table 2
Year effect on the response of sorghum to N and P fertilization

Location and year	Grain yield (kg ha ⁻¹)	Stover yield (kg ha ⁻¹)	TBY* (kg ha ⁻¹)	TKW** (g)	Panicle weight (kg ha ⁻¹)
Mersa					
1997	2479	19280	22403	26	3123
1998	2554	9384	12786	29	3402
F-test	NS	**	**	**	*
Chefa					
1998	4193	8834	18319	30	5201
1999	5407	18319	29970	36	7322
F-Test	**	**	**	**	**
Kobo					
1998	3144	4680	12074	26	4183
1999	3467	3980	12112	27	4383
F-Test	**	**	NS	**	NS

*TBY = Total biomass yield; **TKW = Thousand-kernel weight; NS = Non significant

Grain yield

The responses of the grain yield to nitrogen and phosphorus fertilization in each experimental year and the combined data are demonstrated in Fig. 2 and Table 3, respectively. The results revealed an impressive significant response to N, a variable, inconsistent response to P and a non-significant response to N and P interaction. In both the individual years and the combined analyses N fertilization significantly ($P < 0.01$) increased grain yield in linear responses in all years at all locations, except in 1998 at Mersa, where the highest N rate had a pulling down effect (Fig. 2a-c and Table 3). These results are in general accordance with the results of several studies, which showed the positive response of sorghum grain yield and other cereals to nitrogen fertilization in semi-arid areas (Myers, 1978; Korentajer and Berliner, 1988; Hibberd et al., 1991; Holford et al., 1997). In the combined data the highest N rate of 69 kg ha^{-1} significantly ($P < 0.01$) enhanced grain yield to the order of 29%, 17% and 35% over the nil N treatment at Mersa, Chefa and Kobo, respectively.

Responses of grain yield to P fertilization were variable between seasons and sites, P fertilization had significant ($P < 0.01$) positive effects only in 1998 at Mersa and in 1999 at Chefa. At Kobo, P application had no significant ($P < 0.05$) effect on grain yield (Fig. 2d-f). Similarly, in the combined data P fertilization had significant linear ($P < 0.01$) and quadratic ($P < 0.01$) positive effects only at Mersa and Chefa (Table 3). Responses to P application were small and inconsistent even in responsive locations, ranging from 7 to 17% relative to the control. The poor response to P fertilization at Mersa and Chefa and the total absence of a response at Kobo is possibly due to the high initial soil P status of the research fields (Table 1). The lack of response to P fertilization due to high initial soil P status was also reported elsewhere (Hons et al., 1986; Pala et al., 1992), supporting the present findings.

Grain yield was most closely correlated to panicle weight at all locations with correlation (r) values ranging from 0.91 to 0.99, and to total aboveground biomass with correlation (r) values ranging from 0.87 to 0.95. The highly positive correlations indicate that the increase in panicle weight and total aboveground biomass resulted in increased grain yield, which could be explained by increased number of seeds per panicle and increased photosynthetic area, respectively. Similar results regarding increased grain yield as a result of increased aboveground biomass, leaf area index and panicle number under increased N fertilization were also reported by Nielsen and Halvorson (1991). It has also been indicated that the positive response of grain yield to N fertilization in semi-arid areas is the result of increased water use efficiency achieved through deeper rooting, as well as a reduction in soil evaporative loss through a decrease in the solar irradiation reaching the soil surface due to the increased aboveground biomass (Korentajer and Berliner, 1988; Nielsen and Halvorson, 1991; Shamudzarira, 1994). Evidence from studies in semi-arid areas on maize also indicated that N fertilization increased both shoot and root growth, resulting in a large increase in water use efficiency (Shamudzarira, 1994). This result emphasizes the important role that fertilizer could play in improving sorghum production in the water-limited rainfed systems of Welo.

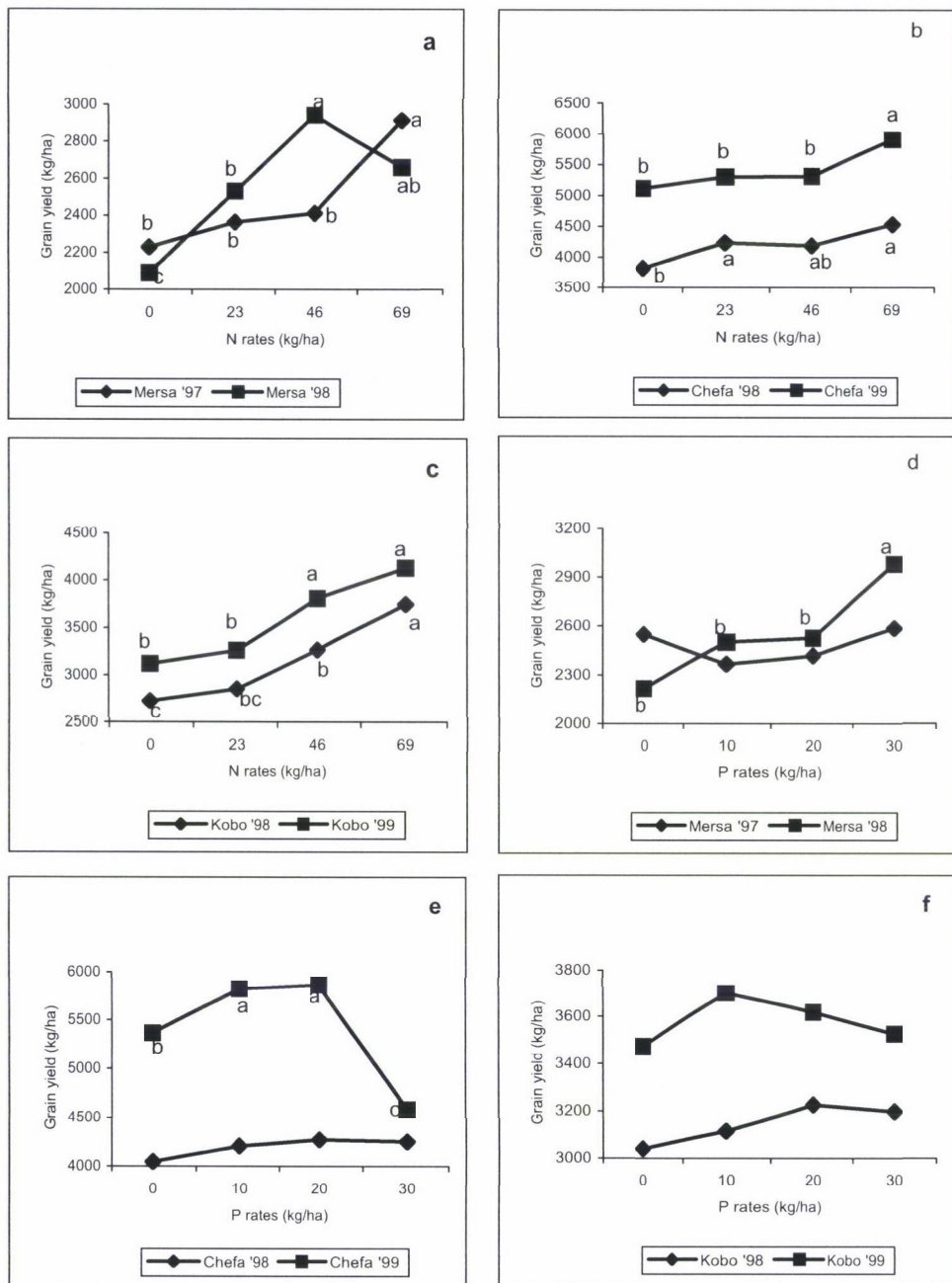


Fig. 2. Sorghum grain yield response to N and P fertilization at the three locations in each individual experimental year. Within the panels (a-f) grain yield data points accompanied by different lowercase letters are significantly different at the 0.05 probability level. Grain yield response lines whose data points are not accompanied by letters indicate non-significant differences in the data points

Table 3

Response of sorghum grain and stover yield to nitrogen and phosphorus fertilizer applications at three semi-arid areas in Welo, Ethiopia

Comparison	Grain yield (kg ha ⁻¹)			Stover yield (kg ha ⁻¹)		
	Mersa	Chefa	Kobo	Mersa	Chefa	Kobo
N rates (kg ha ⁻¹)						
0	2158c	4466c	2872c	(12820b)	12426b	4097b
23	2447b	4768b	3007c	(13230b)	14075a	4001b
46	2675ab	4748b	3470b	(15010a)	13262ab	4420ab
69	2785a	5219a	3872a	(16267a)	14542a	4802a
F-test						
Rate	**	**	**	(**)	*	**
Linear	**	**	**	(**)	*	**
Quadratic	NS	NS	NS	(NS)	NS	NS
P rates (kg ha ⁻¹)						
0	2380b	4705b	3205	(14098)	13416	4281
10	2432b	5014a	3348	(14267)	13121	44269
20	2471b	5066a	3363	(13627)	14345	4344
30	2781a	4416c	3305	(15336)	13423	4426
F-test						
Rate	*	**	NS	(NS)	NS	NS
Linear	**	*	NS	(NS)	NS	NS
Quadratic	NS	**	NS	(NS)	NS	NS
N × P	NS	NS	NS	(NS)	NS	NS
CV (%)	18.7	8.9	16.2	(15.3)	18.5	18.5

Means within a column followed by different letter(s), for each element, are significantly different at the 5% level of probability using LSD. Bracketed figures refer to air-dry weight. * and ** denote significant values at $P<0.05$ and $P<0.01$; NS denotes non-significant values; *TBY = Total biomass yield; **TKW = Thousand-kernel weight

Stover yield

The effect of N and P fertilization on sorghum stover yield is shown in Table 3. Stover yield was predominantly and positively affected by N application in significant ($P<0.01$ and 0.05) linear responses. The application of 46 and 69 kg N ha⁻¹ at Mersa, 23 and 69 kg N ha⁻¹ at Chefa and 69 kg N ha⁻¹ at Kobo gave the highest significant stover yields. These represented increases of 17–27%, 13–17% and 17% relative to the control. Stover yield was not responsive to P fertilization and N and P interactions across all sites.

Total aboveground biomass yield

Total aboveground biomass yield rose significantly in linear ($P<0.01$) responses with incremental rates of N fertilizer (Table 4). The two highest N rates at Mersa and Kobo and the highest rate at Chefa gave the highest significant total aboveground biomass yield. Increases in total aboveground biomass yield were in the order of 17–27% at Mersa, 17% at Chefa and 16–22% at Kobo over the nil N treatments. This agrees with the findings of Hons et al.

(1986) who reported an increase in total aboveground biomass yield with nitrogen application in sorghum. P fertilization had a significant ($P<0.05$) positive effect on total aboveground biomass yield only at Mersa, where 30 kg P ha⁻¹ gave the highest significant ($P<0.05$) value. Total aboveground biomass yield was not responsive to P application at Chefa and Kobo. No significant N and P interaction effects on total aboveground biomass yield were observed at any of the sites (Table 4).

Panicle weight

At all sites, significant linear ($P<0.01$) responses to N fertilization were obtained for panicle weight (Table 5). Panicle weight was significantly enhanced by 27% at Mersa, 16% at Chefa and 32% at Kobo with the application of 69 kg N ha⁻¹ over the nil N treatments. P application significantly increased panicle weight in quadratic ($P<0.01$) response only at one of the three sites. The application of 10 and 20 kg P ha⁻¹ gave significantly higher panicle weight, while the highest rate of 30 kg P ha⁻¹ depressed it. Panicle weight did not respond to the interaction effect of N and P.

Table 4
Response of sorghum total biomass yield to nitrogen and phosphorus fertilizer applications at three semi-arid areas in Welo, Ethiopia

Comparison	Total biomass yield (kg ha ⁻¹)		
	Mersa	Chefa	Kobo
N rates (kg ha ⁻¹)			
0	15723b	22429c	11013b
23	16297b	24444ab	11198b
46	18413a	23461bc	12728a
69	19946a	26244a	13432a
F-test			
Rate	**	**	**
Linear	**	**	**
Quadratic	NS	NS	NS
P rates (kg ha ⁻¹)			
0	17206b	23726	11993
10	17433ab	24215	12122
20	16820b	25377	12385
30	18918a	23260	11871
F-test			
Rate	*	NS	NS
Linear	NS	NS	NS
Quadratic	NS	**	NS
N × P	NS	NS	NS
CV (%)	15.4	13.6	15.1

Means within a column followed by different letter(s), for each element, are significantly different at the 5% level of probability using LSD. * and ** denote significant values at $P<0.05$ and $P<0.01$; NS denotes non-significant values.

Thousand-kernel weight

N application had a significant ($P<0.01$) effect on thousand-kernel weight only at one of the three sites, where 23 and 46 kg N ha⁻¹ gave the highest significant values (Table 5). P fertilization, on the other hand, resulted in significant linear ($P<0.01$) and quadratic ($P<0.01$) increases in thousand-kernel weight at two of the three sites. The application of P at rates of 20 and 30 kg ha⁻¹ at Mersa and 10 and 20 kg ha⁻¹ at Chefa significantly increased kernel weight. There was a positive significant ($P<0.05$) N and P interaction effect on kernel weight at only one of the three sites. The experiments were exposed to an extended dry spell from flowering onwards, suggesting that grain filling may not have been successfully accomplished and was thus unable to express nitrogen treatment effects. Myers (1978) also reported the absence of a response to fertilization in kernel weight due to moisture stress during grain filling.

Table 5

Response of sorghum panicle weight and thousand-kernel weight to nitrogen and phosphorus fertilizer applications at three semi-arid areas in Welo, Ethiopia

Comparison	Panicle weight (kg ha ⁻¹)			1000-kernel weight (g)		
	Mersa	Chefa	Kobo	Mersa	Chefa	Kobo
N rates (kg ha ⁻¹)						
0	2902c	5914b	3761c	26.9b	33.6	27.1
23	3067bc	6184b	3919c	28.3a	33.3	26.0
46	3403ab	6102b	4494b	28.2a	33.0	26.5
69	3679a	6848a	4958a	27.7ab	33.1	26.9
F-test						
Rate	**	*	**	**	NS	NS
Linear	**	**	**	NS	NS	NS
Quadratic	NS	NS	NS	**	NS	NS
P rates (kg ha ⁻¹)						
0	3109	6222ab	4211	27.2b	32.2b	26.3
10	3167	6589a	4314	27.4ab	33.6a	26.8
20	3194	6565a	4332	28.3a	34.0a	26.8
30	3582	5671b	4276	28.2a	33.3ab	26.6
F-test						
Rate	NS	**	NS	*	**	NS
Linear	*	NS	NS	**	*	NS
Quadratic	NS	**	NS	NS	**	NS
N × P	NS	NS	NS	NS	*	NS
CV (%)	20.7	17.2	14.7	5.6	4.9	7.3

Means within a column followed by different letter(s), for each element, are significantly different at the 5% level of probability using LSD. * and ** denote significant values at $P<0.05$ and $P<0.01$; NS denotes non-significant values.

Conclusions

The general misconception in fertilizer application in semi-arid areas is that the use of mineral fertilizers merely increases crop yield. This misconception has led to either no use of mineral fertilizers or the application of lower rates in semi-arid areas. However, the present findings in the semi-arid areas of Welo revealed that the use of higher rates of N (as high as 69 kg ha^{-1}) increased sorghum grain yield and yield components. The data on grain yield and yield components from the three sites showed marked and consistent responses to the application of N fertilizer to grain sorghum, but an inspection of the data suggest that maximum yields were not reached in the rates of N application ranging from 0 to 69 kg N ha^{-1} . At all three sites, grain yields appear to be still increasing at the highest rate of applied N (69 kg ha^{-1}). Rates higher than 69 kg N ha^{-1} would, therefore, be needed to ensure maximum biological yields.

In contrast, P fertilization had only a small effect on sorghum yield and yield components and the responses were inconsistent and variable, because of the high soil P status of the research sites. Hence, before embarking on any conclusion future studies should investigate sorghum responses to P application on farms where the soil P status would possibly be low.

The results of these studies did not support the blanket recommendation of 41 and 20 kg ha^{-1} N and P fertilizers, respectively, currently in use. Based on the results the N rate of the blanket recommendation, 41 kg N ha^{-1} , is suboptimal and that of P, 20 kg ha^{-1} , does not apply for all areas in Welo.

Generally, the results of these studies show that soil fertility management cannot be ignored if the productivity of sorghum is to be improved in semi-arid areas in the short term.

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EFFECTS OF NITROGEN LEVELS AND CROP ARRANGEMENTS ON THE INCIDENCE OF *MEGALUROTHRIPS SJOSTEDTI* TRYBOM AND *MARUCA VITRATA* FAB IN MAIZE/COWPEA INTERCROP

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Four nitrogen levels (0, 20, 30 and 60 kg N/ha) and five crop arrangements were evaluated for their effects on the incidence of *Megalurothrips sjostedti* and *Maruca vitrata* and on the grain yield of maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* Walp). The crop arrangements were one row of maize alternating with one row of cowpea (1:1 inter-row alternate arrangement), one row of maize alternating with two rows of cowpea (1:2 inter-row alternate arrangement) and two stands of maize alternating with two stands of cowpea along the same row (2:2 intra-row alternate arrangement). The other two crop arrangements were the sole maize and sole cowpea control treatments. Nitrogen levels were assigned to the main plots while crop arrangements were assigned to the sub-plots in a split-plot design. Populations of *Megalurothrips sjostedti* and *Maruca vitrata* were significantly affected by crop arrangements. In most cases the 1:1 inter-row alternate arrangement gave the lowest population of these insects. The interaction effects of nitrogen levels and crop arrangements were significant in the case of both thrips and pod borers (*Maruca vitrata*). The yields of both maize and cowpea were affected significantly by the crop arrangements, with sole maize and sole cowpea recording the highest grain yields.

Key words: nitrogen level, crop arrangement, maize/cowpea, *Megalurothrips sjostedti* Trybom, *Maruca vitrata* Fab.

Introduction

The agronomic advantages of mixed cropping are well known, particularly from work carried out in the tropics. There has, however, been an unfortunate dearth of scientific input from entomologists and pathologists despite the recognition accorded insects and diseases as major limiting factors to increases in yields in most tropical crops. It appears from the limited information available that intercropping frequently reduces insect herbivore infestations on the component crops. One reason cited for the wide-scale adoption of mixed cropping in marginal farming is the low incidence of pests (Nickel, 1973).

Various factors are likely to be involved in pest regulation in intercropping, including increased parasitoid, predator populations, the availability of alternative prey, and decreased colonisation and reproduction in the pests. Other factors likely to be involved include chemical repellency, masking and feeding inhibition by odours from non-host plants. The prevention of immigration and optimum synchrony between pests and their natural enemies are also likely factors (Bhatnagar and Davies, 1981). For example, unsprayed cowpea was less subject to insect damage when intercropped with sorghum than

sole cropped cowpea (Raheja, 1977). Similarly, okra (*Abelmoschus esculentus* L. and Moench.) appears to be a useful diversionary crop for flea beetles of the *Podagrica* species attacking cotton (*Gossypium* species) in Nigeria (Usenbo, 1976).

The acquisition of quantitative information about intercrops, with equal emphasis being given to all components, is a pre-requisite of both basic and applied work in insect-plant interactions within intercrops. Such information could form a basis for the development of effective pest management strategies in intercropping, thereby combating one of the major constraints to food production. Intercropping will undoubtedly continue for some considerable time on subsistence farms. Among the various intercropping systems in Nigeria, maize/cowpea intercrop is the predominant mixture in peasant farming communities across almost all agro-ecological zones (Norman, 1973).

In view of the fact that cowpea has an array of insect pests that require the frequent application of insecticides for optimum yields, there is a need to develop a package that is compatible with the circumstances of the small-scale farmers who produce the bulk of the staple food consumed in Nigeria. This is because the subsistence farmer lacks the technology, money and inputs to apply the most cost-effective control measures.

The objective of this work was to look into ways of manipulating maize and cowpea arrangements so that little or no insecticide control is necessary in peasant farming communities, at the same time achieving acceptable yield levels for the two crops.

Materials and methods

A field experiment was conducted during the 1997 cropping season at Badeggi (9° 45'N, 6° 07'E) in the Southern Guinea Savannah zone of Nigeria. The weather and climatic conditions of the area during the season of the experiment are shown in Table 1. There were four levels of nitrogen (0, 20, 30 and 60 kg N/ha) and five crop arrangements. The crop arrangements were

(a) One row of maize alternating with one row of cowpea giving 41,500 maize plants/ha and 40,000 cowpea plants/ha (1:1 maize/cowpea alternate inter-row arrangement)

(b) One row of maize alternating with two rows of cowpea with 40,000 maize plants/ha and 50,000 cowpea plants/ha (1:2 maize/cowpea alternate inter-row arrangement).

(c) Two stands of maize alternating with two stands of cowpea along the same row giving 52,500 maize plants/ha and 45,000 cowpea plants/ha (2:2 maize/cowpea alternate intra-row arrangement).

(d) Sole cowpea (90,000 cowpea plants/ha)

(e) Sole maize (75,000 maize plants/ha)

The land was ploughed and harrowed. The treatments were laid out in a split-plot design with three replicates. Each replicate measured 4 m × 71 m. Nitrogen levels were assigned to the main plots, measuring 4 m × 17 m, while the five crop arrangements were assigned to the split-plots, measuring 4 m × 3 m. The two crops were planted simultaneously on 1st August, 1997. The spacing adopted was 25 cm and 30 cm within rows for cowpea and maize, respectively. The inter-row spacing was 50 cm. Basal application of fertilizer to maize was done at two weeks after planting, and the crop was top dressed before tasselling. The field was kept weed-free throughout the growing season. Crop vigour scores were recorded using a 1–9 scale as follows.

1 = Plants very weak and stunted

3 = Plants less vigorous than normal

5 = Plants intermediate or normal

7 = Vigorous

9 = Extra vigorous.

Table 1
Meteorological data during the period of the experiment (1997)

Month	Rainfall (cm)	Temperature (°C)		Relative humidity (%)	
	Mean	Min.	Max.	10.00 am	4.00 pm
July 1–10	3.7	21.8	29.0	79.6	59.6
11–20	5.2	22.7	30.2	79.4	66.9
21–30	6.8	20.8	30.3	82.2	73.4
Aug 1–10	8.8	19.9	28.7	84.4	75.3
11–20	7.5	19.5	19.1	82.8	73.1
21–30	9.8	21.0	28.5	78.6	87.6
Sept. 1–10	3.9	23.9	29.5	81.9	78.2
11–20	8.1	18.9	29.4	81.6	58.7
21–30	3.8	18.6	28.1	80.5	63.5
Oct. 1–10	31	20.0	31.6	77.9	68.7
11–20	0	19.9	30.6	76.9	68.3

Thrips were sampled by collecting 5 fallen flowers per plot and immersing them in water. The flowers were dissected and the number of the thrips was recorded. This was done at 7 and 8 WAP (weeks after planting). For the cowpea pod borers, *Maruca vitrata*, the number of adult emergence holes (often covered by frass) were recorded on 25 pods (5 pods/plant) per plot. Maize and cowpea grain yields were also recorded. All the data collected were subjected to analysis of variance (ANOVA) and the means were separated using Duncan's Multiple Range Test (DMRT). The land equivalent ratio (LER), which is the relative yield total, was calculated mathematically as

$$\text{LER} = \frac{\text{intercrop yield of maize}}{\text{sole crop yield of maize}} + \frac{\text{intercrop yield of cowpea}}{\text{sole crop yield of cowpea}}$$

An LER greater than 1.0 implies that for that particular crop combination, intercropping yielded more than growing the same number of stands of each crop as sole crops. An LER of less than 1.0 implies that the intercropping was less beneficial than sole cropping.

Results and discussion

The crop vigour was significantly affected ($P < 0.05$) by Nitrogen levels, the highest vigour being recorded at 60 kg N/ha and the lowest vigour in the zero nitrogen treatment (Table 2). Crop arrangements, however, had no significant effect ($P > 0.05$) on vigour. This trend was not unexpected because nitrogen is a vital element required by most cereal crops for growth. Goldsworthy (1967) reported that maize responded to the application of nitrogen fertilizer at a recommended rate of 65 kg N/ha for maximum yield. Nitrogen levels had no significant effect ($P > 0.05$) on thrips abundance; however, crop arrangements significantly affected the number of thrips (Table 3a). The maize/cowpea 1:1 alternate inter-row arrangement gave the lowest population of thrips compared to the 1:2 (maize:cowpea) alternate inter-row ratio and the 2:2 alternate intra-row ratio (i.e. two stands of maize: two cowpea stands along the same row) (Table 3a). The fact that the lowest thrips population was recorded in the 1:1 maize/cowpea alternate row arrangement may be due to the high number of maize plants found in this treatment, which serve as non-host plants and at the

same time create a barrier to the free movement of the thrips. In a millet/groundnut intercrop, Umaru et al. (1998) observed the lowest aphid population in 1:1 millet/groundnut (*Pennisetum glaucum* (L.) R. Br./*Arachis hypogea* L.) alternate inter-row arrangements compared to all the other crop arrangements used.

The significantly ($P < 0.05$) highest number of thrips was recorded in the sole cowpea treatment (Table 3a). This could be due to the greater available space and the greater abundance of the host crop, which are likely to increase the number of thrips. The interaction effect of nitrogen levels and crop arrangements was significant (Table 3b). At a maize/cowpea alternate inter-row ratio of 1:1, there was no significant increase in the number of thrips with increasing nitrogen levels. This is probably due to the fact that nitrogen boosts maize growth which will consequently enhance leaf sizes, height and freshness. The result of this is that more coverage of space is achieved, thus suppressing the movement of the thrips and preventing there being more thrips with increasing nitrogen levels. Similar interaction trends were observed for the 1:2 maize/cowpea alternate inter-row ratio and the 2:2 alternate intra-row ratio.

In the case of *Maruca vitrata* a similar trend to that found for thrips was observed, where nitrogen levels had no significant effect on the number of *Maruca vitrata* but crop arrangements had a significant effect (Table 4a). The sole cowpea treatment again had the highest number of *Maruca vitrata*. The reasons put forward to explain the trends in thrips numbers can also be employed for the trends in *Maruca vitrata*. There was a significant increase in the number of *Maruca vitrata* in all the crop arrangements when the nitrogen level was increased from 0 kg N/ha to 20 kg N/ha (Table 4b). A further increase in the nitrogen level from 20 kg N/ha to 30 kg N/ha did not show a significant increase in the number of *Maruca vitrata* at a maize/cowpea alternate inter-row ratio of 1:2 (Table 4b). The highest number of *Maruca* was observed in the sole cowpea treatments, especially at 20 kg N/ha and 30 kg N/ha (Table 4b).

Table 2
Effect of nitrogen levels and crop arrangements on the crop vigour of maize at 8 WAP

Nitrogen levels kg N/ha (N)	Vigour Score
0	3.4c
20	7.6b
30	8.4a
60	8.5a
SE \pm	0.31
Crop arrangements (C)	
1:1 Maize:cowpea inter-row ratio	7.0
1:2 Maize:cowpea inter-row ratio	6.8
2:2 Maize:cowpea intra-row ratio	6.9
Sole maize	7.0
SE \pm	0.17
Interaction (N \times C)	NS

WAP: Weeks after planting; NS: Non-significant

Table 3a

Effect of nitrogen levels and crop arrangements on the abundance of thrips at 7 and 8 WAP

Nitrogen levels kg N/ha (N)	7 WAP	8 WAP
0	16.8	15.3
20	27.3	18.6
30	17.0	21.2
60	21.8	71.2
SE±	1.5	1.7
Crop arrangements (C)		
1:1 Maize:cowpea inter-row ratio	15.4b	15.1c
1:2 Maize:cowpea inter-row ratio	17.0b	17.7b
2:2 Maize:cowpea intra-row ratio	17.7b	17.8b
Sole maize	22.7a	21.5a
SE±	0.48	0.62

Interaction (N x C) ****

Table 3b

Interaction effect of nitrogen levels and crop arrangements on the population of thrips

Crop arrangements	Nitrogen levels (kg N/ha)			
	0	20	30	60
1:1 Maize:cowpea inter-row ratio	16.7ade	13.7e	15.0de	16.3ade
1:2 Maize:cowpea inter-row ratio	16.0de	17.0c	15.7de	19.3bc
2:2 Maize:cowpea intra-row ratio	17.3cd	16.7ad	15.0de	21.7b
Sole maize	17.0cd	21.7b	22.3b	29.7a

SE±: 1.30

Table 4a

Effect of nitrogen levels and crop arrangements on the abundance of cowpea pod borer *Maruca vitrata*

Nitrogen levels kg N/ha (N)	Population of pod borer/plot
0	13.8
20	24.4
30	20.3
60	17.7
SE±	1.86
Crop arrangements (C)	
1:1 Maize:cowpea inter-row ratio	16.17c
1:2 Maize:cowpea inter-row ratio	18.0b
2:2 Maize:cowpea intra-row ratio	17.5b
Sole cowpea	23.6a
SE±	0.72
Interaction (N x C)	**

Table 4b

Interaction effect of nitrogen levels and crop arrangements on the population of cowpea pod borer

Crop arrangements	Nitrogen levels (kg N/ha)			
	0	20	30	60
1:1 Maize:cowpea inter-row ratio	13.3e	20.7bc	15.7de	15.0de
1:2 Maize:cowpea inter-row ratio	15.3 e	20.3bcd	20.3bcd	16.0cbe
2:2 Maize:cowpea intra-row ratio	14.0e	22.0b	17.0cde	27.0cde
Sole cowpea	12.7e	30.7a	28.3a	22.7b

SE±: 1.62

These trends could be explained by the fact that in the intercropped treatments the presence of maize plants arranged alternately between the rows or along the rows created a barrier to the free movement of the thrips as compared to sole cowpea treatments that have no barriers. Hence, more thrips are recorded in sole cowpea treatments than in any of the intercropped treatments. In Nigeria unsprayed cowpea was less vulnerable to attack by insects when intercropped with *Sorghum bicolor* (L.) Moench than when sole cropped (Uvah, 1978; Raheja, 1977). It is also possible for an insect pest to exhibit an avoidance reaction, being caused to leave the vicinity of the mixture when it lands on a non-host intercropped with the host. The dispersal of both the adult and larval stages of insect pests may be impeded where hosts and non-host crops are grown together. The non-host crops may offer a barrier to the dispersal of insects into and within the host crop habitat. All these mechanisms could lead to reduced insect incidence in intercropping as compared to monocropping. Taylor (1977) observed that *Maruca vitrata* attacked cowpea flowers less when cowpea was intra-rowed with maize. The significance of nitrogen levels, apart from enhancing maize yields, is to induce vigour, which can be translated in terms of height, sizes of leaves and other plant parts, and freshness. If these are enhanced maize plants intercropped with cowpea provide better cover and create barriers to the free movement of the insects. Hence the insect population in intercropping is reduced.

Grain yields were significantly affected ($P < 0.05$) by the crop arrangements, with sole maize recording the highest grain yields. The maize/cowpea intra-row ratio of 2:2 gave higher maize grain yields than the other two crop arrangements (Table 5). This is possibly due to the fact that there was more efficient utilization of nutrients, space and light due to the keen competition occasioned by the higher number of both maize and cowpea plants. The interaction effect of nitrogen levels and crop arrangements was not significant (Table 5). Cowpea grain yields were not significantly ($P > 0.05$) affected by the nitrogen levels but were significantly ($P < 0.05$) affected by the crop arrangements (Table 6). Sole cowpea gave significantly ($P < 0.05$) higher grain yields. However, the differences in yield between all the intercropped arrangements were not significant (Table 6). This may be due to the fact that cowpea growing beneath the maize is subjected in all cases to nearly the same growth conditions due to the micro-climate created by the presence of maize. The land equivalent ratio (LER), shown in Tables 5 and 6, generally indicates that there is an advantage in the crop arrangements used, as none of the crop arrangements had an LER value of less than 1.0.

Conclusions

It can be deduced from these studies that given the present situation of peasant farmers in a developing country, where farmers have few pest control

options compared to the more advanced countries because they often lack the technology, money and expertise to exercise the most cost-effective control strategies, farmers faced with insect pest problems should intensify efforts to practise mixed cropping. This is evident from the results obtained, where the maize/cowpea alternate inter-row ratio of 1:1 exhibited the lowest pest populations of thrips and pod-borers. As a result, the intercropping of cereals with cowpea will make it possible to reduce spraying regimes in cowpea and lower the costs of production. The activities of natural enemies are also enhanced in crop mixtures. These factors combine to ensure an integrated pest management approach.

Table 5
Effect of nitrogen levels and crop arrangements on the maize grain yield (kg/ha)

Nitrogen levels kg N/ha (N)	Grain yield (kg/ha)	LER
0	825.7d	1.0
20	1347.9c	1.0
30	1791.6b	1.0
60	2196.5a	1.0
SE±	272.8	
Crop arrangements (C)		
1.:1 Maize:cowpea inter-row ratio	1075.7c	1.1
1:2 Maize:cowpea inter-row ratio	1056.2c	1.0
2:2 Maize:cowpea intra-row ratio	1432.6b	1.2
Sole maize	2597.1a	1.0

SE: ±236.2

Interaction (N × C): NS

LER: Land equivalent ratio; NS: Non-significant

Table 6
Effect of nitrogen levels and crop arrangements on the cowpea grain yield (kg/ha)

Nitrogen levels kg N/ha (N)	Grain yield (kg/ha)	LER
0	243.9	1.1
20	235.3	1.0
30	260.1	1.0
60	262.1	1.0
SE±	24.1	
Crop arrangements (C)		
1.:1 Maize:cowpea inter-row ratio	226.9b	1.1
1:2 Maize:cowpea inter-row ratio	220.5b	1.0
2:2 Maize:cowpea intra-row ratio	226.0b	1.2
Sole maize	328.1a	1.0

SE±: 22.9

Interaction (N × C) NS

LER: Land equivalent ratio; NS: Non-significant

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PLANT HEIGHT AND HEIGHT OF THE MAIN EAR IN MAIZE (*ZEA MAYS* L.) AT DIFFERENT LOCATIONS AND DIFFERENT PLANT DENSITIES

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The plant height and the height of the main ear were studied over two years in twelve single cross maize hybrids sown at three different plant densities (45, 65 and 85 thousand plants/ha) at five locations in Hungary (Keszthely, Gönc, Gyöngyös, Sopronhorpács, Martonvásár).

The results revealed that plant height and the height of the main ear are important variety traits and are in close correlation with each other. It was found that the hybrids grew the tallest when the genetic distance between the parental components was greatest (Mv 4, Mv 5). The height of the main ear was also the greatest in these hybrids, and the degree of heterosis was highest (193% for plant height, 194% for the height of the main ear). The shortest hybrids were those developed between related lines (Mv 7, Mv 11). In this case the heterosis effect was the lowest for both plant height (128%) and the height of the main ear (144%).

The ratio of the height of the main ear to the plant height was stable, showing little variation between the hybrids (37–44%).

As maize is of tropical origin it grows best in a humid, warm, sunny climate. Among the locations tested, the Keszthely site gave the best approximation to these conditions, and it was here that the maize grew tallest. The dry, warm weather in Gyöngyös stunted the development of the plants, which were the shortest at this location.

Plant density had an influence on the plant size. The plants were shortest when sown at a plant density of 45,000 plants/ha, and the main ears were situated the lowest in this case. At all the locations the plant and main ear height rose when the plant density was increased to 65,000 plants/ha. At two sites (Gönc and Sopronhorpács) the plants attained their maximum height at the greatest plant density (85,000 plants/ha). In Keszthely there was no significant difference between these two characters at plant densities of 65 and 85 thousand plants/ha, while in Gyöngyös and Martonvásár the greatest plant density led to a decrease in the plant and main ear height.

The year had a considerable effect on the characters tested.

Key words: maize hybrid, plant height, height of main ear, location, plant density, year

Introduction

In experiments conducted by Daniel and Bajtay (1975) plant height exhibited significant genotypic variation, with a high degree of heritability ($h^2=0.813$).

The location has a substantial effect on plant height and the height of the main ear (El-Sherbieny et al., 1991). There may be considerable differences in this respect between maize plants grown in different environments even within the same variety (Wu, 1988).

The genotype \times year interaction was not significant for plant height and the height of the main ear in experiments carried out by Russell (1976).

The plant height and the height of the main ear are also influenced by the production technology. Illumination conditions depend on the plant density and exert an effect on plant size (Andrejenko and Kuperman, 1961; Obilana and Hallauer, 1974). In plant density experiments maize was found to grow ever higher as the degree of mutual shading increased (Hozumi et al., 1955). Plant height and the height of the main ear also increased at greater plant density in the experiments of Hassan (2000). Similar results were reported by Pucaric (1974), who found that the plant height of the hybrids tested increased linearly with an increase in plant density. The height of the main ear was also in linear correlation with the plant density for all the hybrids. The mean increase was 2.17–2.39 cm for each thousand-plant increase in density. When selecting for yield increase, Moll and Kamprath (1977) observed a slight increase in the plant height–main ear height difference (top height) at different plant densities.

Materials and methods

Twelve single cross hybrids were developed (Table 1) from seven Martonvásár inbred maize lines (Table 2) in the maize nursery of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, in 1996.

In 1997–1998 the inbred lines and the twelve hybrids were tested at five locations (Keszthely, Gönc, Gyöngyös, Sopronhorpács, Martonvásár).

The inbred lines were grown at a plant density of 65,000 plants/ha, while the hybrids were evaluated at three plant densities (45, 65 and 85 thousand plants/ha) in a split-plot design with three replications.

The experiments were carried out on chernozem soil in Martonvásár and Gyöngyös and on brown forest soil in Keszthely, Gönc and Sopronhorpács.

Table 1
Scheme of hybrid production (1996)

Lines	HMv 1	HMv 2	HMv 3	HMv 4	HMv 5	HMv 6	HMv 7
HMv 1	–		Mv 10	Mv 12	Mv 9		
HMv 2		–		Mv 1			
HMv 3			–	Mv 2		Mv 4	Mv 5
HMv 4				–	Mv 7		
HMv 5			Mv 8		–	Mv 3	Mv 6
HMv 6						–	
HMv 7						Mv 11	–

Table 2
Certain properties of the inbred lines included in the experiment (1996)

Line	Genetic background	Kernel type	Tasselling (days)	Silking (days)	Plant height (cm)	Height of main ear (cm)
HMv 1	IODENT	Dent	76	78	166	61
HMv 2	IODENT	Dent	81	83	182	62
HMv 3	IODENT	Dent	84	82	189	64
HMv 4	B 37	Dent	77	78	140	48
HMv 5	B 37	Dent	78	79	155	62
HMv 6	MP yellow	Dent	78	79	139	53
HMv 7	MP yellow	Flint, hard-grained	79	79	147	46

The rainfall and temperature data of the five locations during the vegetation period are presented in Figure 1. There were differences between the sites in rainfall supplies, mean temperature and the number of sunshine hours.

The 1997 season was very dry, with less rainfall than the many years' average at all the sites. The greatest quantities of rainfall were recorded in Sopronhorpács (431 mm), Gönc (356 mm) and Keszthely (354 mm), which was sufficient for normal plant development. In Gyöngyös and Martonvásár, however, there was very little rain (271 and 238 mm, respectively), while the mean temperature at these two locations was the highest during the vegetation period, leading to symptoms of atmospheric drought. The environmental conditions during the stalk elongation phase has a substantial influence on the final height of the maize stalks. The rainfall and temperature values during the period of intensive growth (July) thus play an important role in determining the final size of the plants. During this month the greatest rainfall was recorded in Sopronhorpács (167 mm) and Gönc (154 mm), followed by Keszthely (109 mm), Martonvásár (108 mm) and Gyöngyös (101 mm). The distribution of the July rainfall over the month was the most favourable for the plants in Keszthely. The mean temperature during the vegetation period was lowest in Gönc (14.7°C) and highest in Gyöngyös (16°C). The number of sunshine hours was highest in Keszthely (1717 h) and lowest in Gönc (1482 h).

In 1998 there was more rain at all the locations than in the previous year. The highest figures were recorded in Gönc (684 mm), Keszthely (642 mm) and Sopronhorpács (609 mm) and the lowest in Martonvásár (522 mm) and Gyöngyös (473 mm). The distribution of rainfall in July showed a similar pattern, with the highest quantities in Sopronhorpács (167 mm) and Gönc (166 mm). There was only 89 mm rainfall in Keszthely during July, but 130 mm was recorded during the last ten days of June. The rainfall was again low in Gyöngyös (67 mm) and Martonvásár (57 mm). The mean temperature during the vegetation period was highest in Gyöngyös (21.7°C) and again lowest in Gönc (16°C). The highest number of sunshine hours was recorded in Keszthely (1502 h) and the lowest in Gönc (1289 h).

The aim of the experiment was to determine the plant height and the height of the main ear in new hybrids developed from Martonvásár inbred lines. The plant data were recorded in the field. The results were statistically analysed using the Agrobases '99 four-factor ANOVA program.

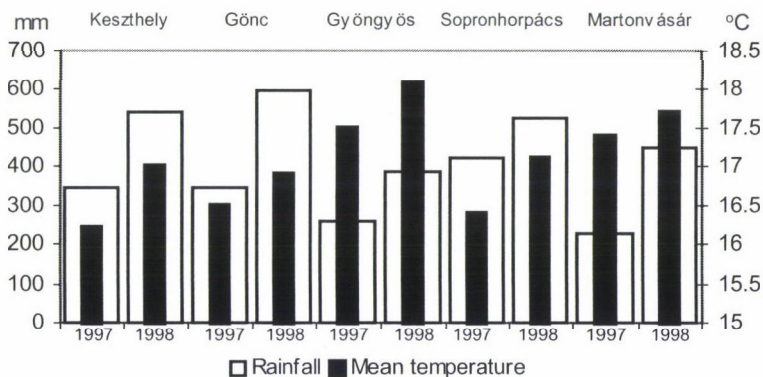


Fig. 1. Rainfall and temperature conditions during the vegetation period at the various locations

Results

The MQ values (mean square deviations) indicate that both characters tested were influenced to the greatest extent by the location, followed by the variety, the year and finally the stand density. An analysis of interactions also showed that the location \times year effect was the most significant. The F values obtained from analysis of variance proved to be significant at the $P=0.1\%$ level for all the factors (Table 3).

Table 3
Table of variance for the four-factor split-plot experiment

Factor	FG	Plant height		Height of main ear	
		MQ	F -value	MQ	F -value
Variety	11	46129.451	2110.33***	11632.658	560.04***
Plant density	2	7186.984	328.79***	4268.359	205.49***
Location	4	93171.128	4262.40***	25212.076	1213.80***
Year	1	22788.445	1042.53***	41168.726	1982.02***
Variety × density	22	338.768	15.50***	156.968	7.56***
Variety × location	44	706.248	32.31***	329.680	15.87***
Variety × year	11	483.831	22.13***	447.065	21.52***
Density × location	8	1600.431	73.22***	669.593	32.24***
Density × year	2	1127.851	51.60***	1046.959	50.40***
Location × year	4	61078.286	2794.22***	13309.927	640.79***
Variety × density × location	88	158.479	7.25***	122.355	5.89***
Variety × density × year	22	213.079	9.75***	131.550	6.33***
Variety × location × year	44	220.083	10.07***	212.350	10.22***
Density × location × year	8	1843.295	84.33***	576.473	27.75***
Variety × density × location × year	88	205.409	9.40***	174.068	8.38***
Error	718	21.859		20.771	

1. Genotypes

Plant height

There were considerable differences in the plant heights of the hybrids tested in the experiments. Hybrids in which there was a wide genetic distance between the parents developed taller stalks. The plant height of hybrids Mv 4 and Mv 5 was greatest, averaged over the two experimental years (262 and 264 cm, respectively). These two hybrids had the same female parent (an Iodent line), while their male parents were Mindszentpusztai sárga (MP yellow) lines. The heterosis effect was 155% and 153%, respectively, for these hybrids. The degree of heterosis was also high (157%) for hybrid Mv 3, which also originated from a cross between genetically distant parents (B 37 × Mp. sárga). The shortest hybrid (Mv 7, 185 cm) was developed from a sib line cross, where the degree of heterosis was the lowest (122%). Hybrid Mv 11, developed from a cross between two Mindszentpusztai sárga lines, had a plant height of 198 cm and a heterosis value of 134%.

Height of main ear

The main ears grew at the highest position in hybrids Mv 4 and Mv 5 (113 and 107 cm, respectively). The main ears of hybrids Mv 3, Mv 9 and Mv 10 were also located at a height of over 100 cm (101, 102 and 103 cm, respectively). Those of Mv 11 (73 cm) and Mv 7 grew at the lowest height. Heterosis was the greatest in hybrids Mv 4 and Mv 5 (194 and 195%, respectively) and was around 180% in hybrids Mv 1, Mv 2 and Mv 3. The lowest degree of heterosis was recorded for hybrids Mv 7 and Mv 11 (Fig. 2).

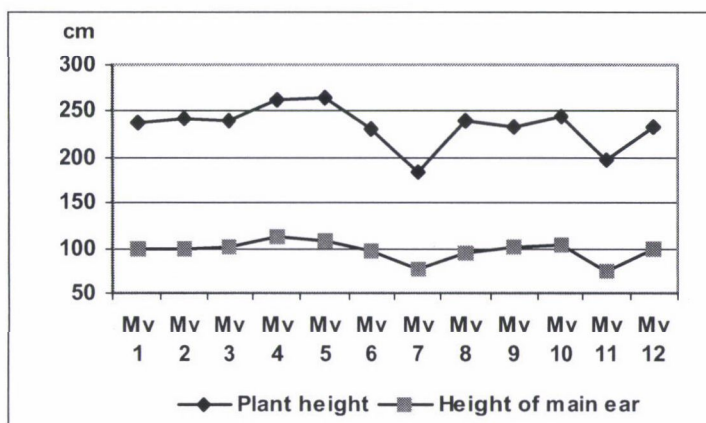


Fig. 2. Plant height and height of the main ear in different genotypes, averaged over 1997–1998

2. Location

Plant height

A comparison of the locations demonstrated significant differences. The maize plants grew tallest in Keszthely (260 cm), followed by Sopronhorpács (246 cm) and Gönc (236 cm), while the shortest plants were found in Martonvásár (219 cm) and Gyöngyös (208 cm).

Height of main ear

The main ear was located at the greatest height in Keszthely (109 cm), followed by Sopronhorpács (106 cm) and Gönc (96 cm). The ears grew lowest on the maize plants in Martonvásár (92 cm) and Gyöngyös (83 cm). The differences between the locations were significant (Table 4).

Table 4

Plant height of the hybrids and height of the main ear at each location, averaged 1997–1998

Location	Plant height (cm)	Height of main ear (cm)
Keszthely	260	109
Gönc	236	96
Gyöngyös	208	83
Sopronhorpács	246	106
Martonvásár	219	92
LSD _{5%}	0.883	0.861

3. Genotype \times location interaction

Plant height

When the genotypes were analysed at each location it was found that plant height is an important, variety-specific trait. At all the locations Mv 4 and Mv 5 had the greatest stalk height (averaging 262 and 264 cm, respectively). The

female parent of both hybrids was the same tall-stalked line (HMv 3). Hybrids in which the female parent was a tall-stalked line (Mv 1, Mv 9, Mv 10) developed tall stalks at all the locations. There were also short-stalked hybrids in the experiment. Those with the shortest stalks at all locations were Mv 7 and Mv 11, in both of which the male and female parents were short-stalked lines (Table 5).

Height of main ear

An analysis of the genotypes showed that the main ears of hybrids Mv 4 and Mv 5 grew higher than those of the other hybrids at all locations except Gönc. The height of the main ear was lowest for Mv 7 and Mv 11 at all the locations (Table 6).

Table 5
Genotype \times location interaction on plant height, averaged over 1997–1998

Hybrids	Keszthely	Gönc	Gyöngyös	Sopronh.	Martonvásár	Mean
Mv 1	264	246	208	247	217	237
Mv 2	278	246	208	252	227	242
Mv 3	264	234	212	259	224	239
Mv 4	294	263	231	281	241	262
Mv 5	293	270	235	275	246	264
Mv 6	255	231	205	243	220	231
Mv 7	208	170	175	191	179	184
Mv 8	269	244	211	246	222	238
Mv 9	255	235	211	245	219	233
Mv 10	269	248	213	262	225	244
Mv 11	218	204	176	206	186	198
Mv 12	251	243	212	240	216	232
Mean	260	236	208	246	219	234
LSD _{5%} genotype \times location				3.060		
LSD _{5%} between any two combinations				7.495		

4. Genotype \times year interaction

Plant height

The two years differed substantially as regards rainfall and temperature. In the wetter year (1998) the plants of all the genotypes grew significantly taller than the mean of the previous year. Averaged over the hybrids the difference between the two years was 9 cm. The greatest difference between the years was recorded for hybrid Mv 6 (18 cm), and a similar difference was found for Mv 5 (16 cm). The smallest difference was recorded for the shortest hybrids (Mv 7: 4 cm, Mv 12: 5 cm) (Fig. 3).

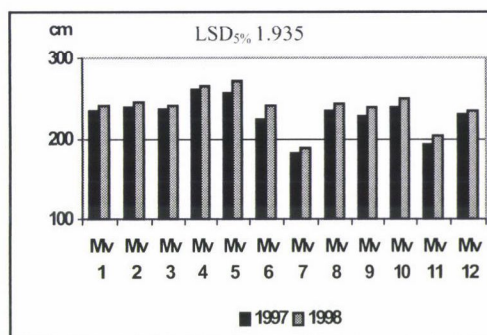
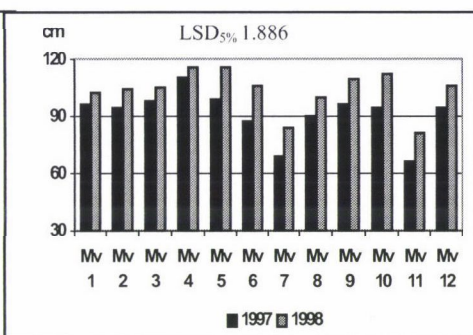
Height of main ear

In 1998 the height of the main ear was on average 12 cm greater than in the previous year. The greatest difference was found for Mv 5 (17 cm), Mv 6 (19 cm) and Mv 10 (18 cm) and the smallest for Mv 3, Mv 4 and Mv 8 (7, 6 and 10 cm, respectively) (Fig. 4).

Table 6

Genotype \times location interaction on height of the main ear, averaged over 1997–1998

Hybrids	Keszthely	Gönc	Gyöngyös	Sopronh.	Martonvásár	Mean
Mv 1	115	106	79	105	90	99
Mv 2	113	102	79	107	92	99
Mv 3	111	96	87	113	99	101
Mv 4	123	114	96	129	104	113
Mv 5	119	102	96	118	102	107
Mv 6	109	91	82	105	96	97
Mv 7	90	66	69	85	74	77
Mv 8	113	93	79	104	86	95
Mv 9	115	102	86	111	98	102
Mv 10	115	102	88	114	97	103
Mv 11	80	76	65	76	73	74
Mv 12	107	103	88	109	92	100
Mean	109	96	83	106	92	97
LSD _{5%} genotype \times location				2.983		
LSD _{5%} between any two combinations				7.306		

Fig. 3. Genotype \times year interaction on plant heightFig. 4. Genotype \times year interaction on height of the main ear

5. Plant density

Plant height

The plants became taller as the plant density increased. The lowest plant height was measured at a density of 45,000 plants/ha and this was significantly different from the values recorded at the other two plant densities. The height of plants grown at densities of 65 and 85 thousand plants per hectare did not differ significantly.

Height of main ear

With an increase in plant density there was also an increase in the height of the main ear, which was in the lowest position (93 cm) on plants grown at a density of 45,000 plants/ha and 6 cm higher (99 cm) in the 65,000 and 85,000 plants/ha treatments, which did not differ significantly from each other (Fig. 5).

6. *Plant density × location interaction*

Plant height

Among the locations, plants grown at a density of 45,000 plants/ha in Keszthely were 10 cm shorter than those grown in denser fields. When the plant density was increased to 65,000 plants/ha the plants reached their maximum height, which did not differ significantly from that of plants grown at the greatest density. In Gönc and Sopronhorpács the plant height increased linearly as the plant density increased and the differences were significant. In Gyöngyös and Martonvásár, on the other hand, the plant height increased to a maximum at 65,000 plants/ha, after which it declined. Significant differences were found between the plant densities, except between densities of 45 and 85 thousand plants/ha in Gyöngyös (Fig. 6).

Height of main ear

In Keszthely the main ear grew at the lowest height on plants grown at a density of 45,000 plants/ha (106 cm), while the main ear was 5 cm higher at plant densities of 65 and 85 thousand plants/ha. The smallest plant density gave values significantly different from those recorded at the two greater plant densities.

In Gönc and Sopronhorpács there was a significant increase in the height of the main ear as the plant density rose. In Gyöngyös and Martonvásár the height of the main ear increased up to a plant density of 65,000 plants/ha, while a further increase in the density to 85,000 plants/ha caused the main ear to grow in a lower position (Fig. 7).

7. *Plant density × year interaction*

Plant height

In 1998 plants in the 45,000 plants/ha treatment grew 5 cm taller on average than in the previous year. Those grown at 65,000 plants/ha were 10 cm taller, while those in the densest stands were only 2 cm taller than in the previous year. The differences were significant.

Height of main ear

The main ears of plants grown at a plant density of 45,000 plants/ha were situated 8 cm higher in 1998 than in 1997. The height of the main ear differed by 14 cm between the two years in the 65,000 plants/ha treatment and by 13 cm in the densest stands (Fig. 8).

The experimental results confirm the findings of El-Sherbieny et al. (1991), who stated that the location had a significant influence on the plant height and on the height of the main ear. These properties of the hybrids examined varied with the growing site, as in the experiments of Wu (1988). In contrast with the conclusions drawn by Russell (1976) the genotype × year effect was found to be significant. The results obtained in the plant density experiment are in agreement with those published by Hozumi et al. (1955), Hassan (2000) and Pucaric (1974).

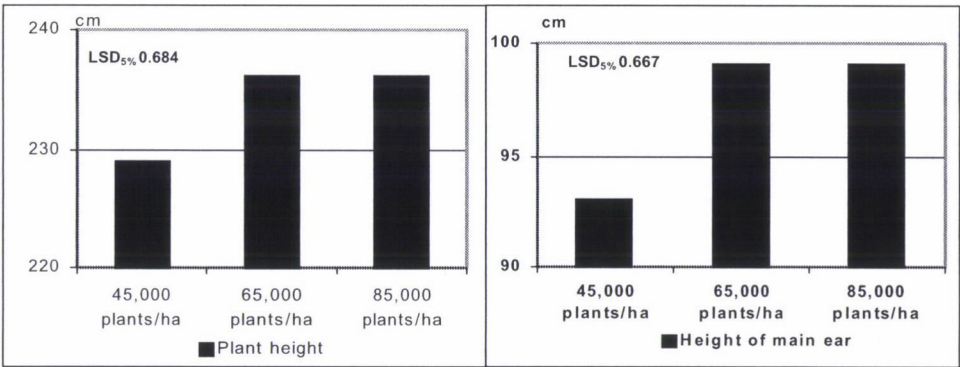


Fig. 5. Plant height and height of the main ear at different plant densities, averaged over 1997–1998

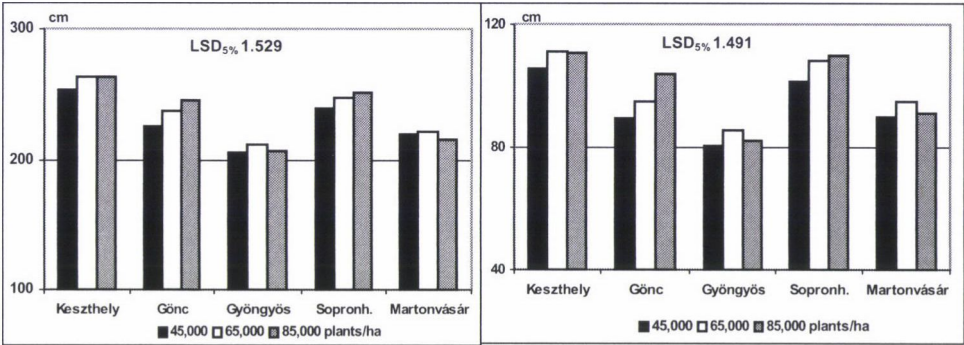


Fig. 6. Plant density \times location interaction on plant height, averaged over 1997–1998

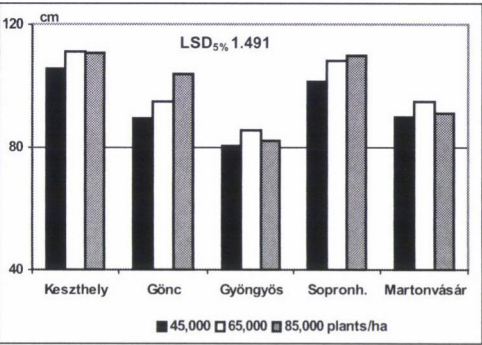


Fig. 7. Plant density \times location interaction on height of the main ear, averaged over 1997–1998

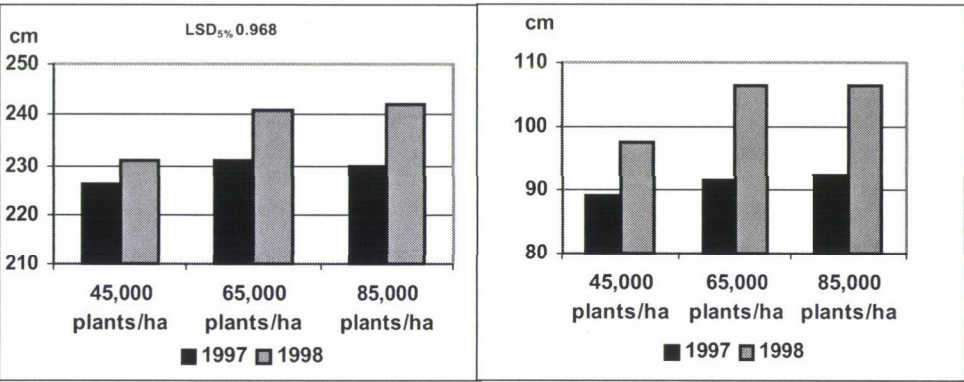
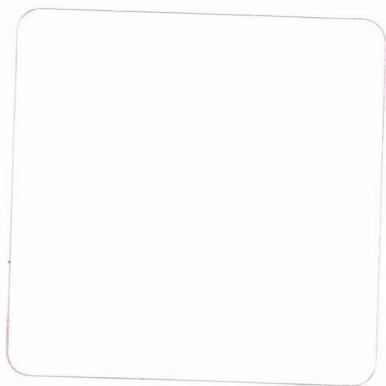


Fig. 8. Plant density \times year interaction on plant height and height of the main ear

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Short communication

FIELD INCIDENCE OF RICE YELLOW MOTTLE VIRUS, GENUS SOBEMOVIRUS ON RICE AND A WEED SPECIES IN THE FIELD IN COTE D'IVOIRE

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Field surveys carried out between 1996 and 1997 in Cote d'Ivoire on weed hosts to detect the occurrence and subsistence of rice yellow mottle virus (RYMV) in nature show that rice and *Echinochloa crus-galli* (Link) harbour the virus. There was consistent detection of RYMV throughout the sampling period in rice samples mostly from the lowland varieties. It is thus evident that RYMV subsists more on rice in nature. This could serve as a source of inoculum for infection to newly transplanted rice in the field.

Key words: field incidence, rice yellow mottle sobemovirus, weed species

Introduction

Rice yellow mottle virus (RYMV), which was first noticed in Kenya, East Africa in 1966 (Bakker, 1974), was reported in West Africa in 1975 (Raymundo and Buddenhagen, 1976). The virus belongs to the sobemovirus group (Seghal, 1981; Hull, 1988). It has reached epidemic proportions in some African countries and is spreading fast in others (Reckhaus and Adamou, 1986; John et al., 1986; Sy, 1994; Abo et al., 1998).

There have been several reports of weeds serving as reservoir hosts of viruses (Howell and Mink, 1977; Givord, 1982; Alegbejo, 1987). Outside the genus *Oryza* some graminaceous species, particularly in the tribe *Eragrostidae*, were found to be systemic hosts of RYMV in Kenya (Bakker, 1974, 1975). Bakker (1974) reported that the virus was probably present on the grass hosts before rice cultivation was introduced and then spread to rice. According to Okioma et al. (1983) and Awoderu (1991) some grasses and wild *Oryza* species which occur abundantly round rice fields are likely to be important reservoirs of the viruses during the off season. RYMV was also detected in *Oryza longistaminata*, a wild species of rice growing in marshy areas and in rice fields in Niger, Mali (John et al., 1984; Sy, 1994), Senegal (Mbodj et al., 1984) and Burkina Faso (Konate, 1995). However, in spite of an intensive search in Kenya and Nigeria for weed hosts of the virus no evidence for the natural occurrence of RYMV in indigenous *Oryza* species or on grasses has been obtained (Bakker, 1974; Rossel, 1986). Hence this study was conducted to establish the weed hosts and subsistence of RYMV in areas where the virus is endemic.

Materials and methods

Monthly surveys were carried out at the irrigation rice scheme at Sakassou and the upland rice ecology at Mbe, Cote d'Ivoire between July 1996 and July 1997. Various weed species and rice with or without virus-like symptoms of RYMV were sampled at random on the bunds, the edges of the fields, within the rice fields and in the vicinity of RYMV-infected plants. Bait plants were placed round the fields and monitored for infection by RYMV. The bait plants were seedlings of Bouake 189, a susceptible check to RYMV. Observations were taken on the predominance of insect vectors of RYMV at the sampled sites. The insects and weed samples collected were identified by the entomologist and weed scientist at WARDA, Bouake, Cote d'Ivoire. The rice and weed samples were analysed by the Enzyme-Linked Immunosorbent Assay (ELISA) technique developed by Clark and Adams (1977) using the Antigen Coated Plate (ACP) form of ELISA devised by Koenig and Paul (1982). Alkaline phosphatase enzyme was conjugated to antiglobulin (Koenig, 1981), and antigen was directly trapped on the microtitre plate. This was detected by a conjugate against the RYMV antibody introduced after the antigen was used. This form of indirect ELISA (Koenig and Paul, 1982) is simple and suitable for virus detection in disease surveys and for unfractionated antiserum (Mowat and Dawson, 1987). The antigen was bound directly to the wells of the microtitre plates. This was followed by addition of the RYMV polyclonal antibody (IgG) which bound to the antigen. The specific antibody was detected by the anti-rabbit IgG enzyme conjugate. The blocking solution contained phosphate- buffered saline and 3% of 99% fat-free milk (Marvel). The ELISA test was performed on the leaves of rice and weeds with a working dilution of 1:10 [0.2 g of leaves in 2 ml of coating buffer plus 2% polyvinyl pyrrolidone (PVP)]. The PVP was used to inhibit the non-specific binding of the conjugate. The leaves were homogenized with a roller press and collected in Eppendorf tubes. The working dilution for both the antibody and conjugate was 1:1000. Each sample was replicated in two wells of the microtitre plate with positive and negative controls for the ELISA test. Finally 100 µl of 0.6 mg/ml 4-nitrophenyl phosphate buffer at pH 9.8 was pipetted into each well of the microtitre plate and incubated at 37 °C for 30 minutes. The colour change was read with a Dynatech ELISA Microreader. Absorbance values (A 405 nm) were accepted as positive when the reading was greater than twice the mean absorbance of the virus-free control sample. The positive values could also be visualized when the colourless substrate p-nitrophenyl-phosphate gave rise to a yellow p-nitrophenol product.

Results and discussion

The results of ELISA on rice and weed samples are presented in Tables 1 and 2. Rice samples were found to be infected by RYMV throughout the sampling period in 1996 and 1997. No infection was detected on the upland rice varieties even though most of the bait plants placed round the fields were infected. This was expected, because most upland varieties are tolerant or resistant to the virus (Thottappilly and Rossel, 1993). The virus was detected only twice in *Echinochloa crus-gavonis* throughout the sampling period between July 1996 and July 1997. No RYMV infection was detected in the other weed samples belonging to the *Cyperaceae*, *Solanaceae* and *Compositae* families. *Leersia haxandra* Sw. was observed to be attacked by *Trichispa sericea* Guerin, a vector of RYMV (Bakker, 1971). The ratooned/volunteer rice was infested by *T. sericea*, and such infested plants showed symptoms of RYMV. Infection of the ratooned/volunteer rice was confirmed by the ELISA test. No infection was detected on *Leersia haxandra* Sw.

Table 1

Results of Enzyme-Linked Immunosorbent Assay (ELISA, A 405 nm) of leaf samples of rice and weeds collected from the rainfed/irrigated rice scheme at Sakassou, Cote d'Ivoire to detect rice yellow mottle virus infection in nature

Plant species and family	1996							1997						
	07	08	09	10	11	12	01	02	03	04	05	06	07	
*Rice (<i>Oriza sativa</i> L.), <i>Gramineae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	
<i>Echinochloa colona</i> Link, <i>Gramineae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Echinochloa crus-pavonis</i> Schultes, <i>Gramineae</i>	-	-	-	-	-	-	-	-	-	-	-	+	+	
<i>Echinochloa pyramidalis</i> Hitchc & Chase, <i>Gr.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Leersia hexandra</i> Sw., <i>Graminae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Imperata cylindrica</i> L., <i>Graminae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Septochloa caerulea</i> Steudel, <i>Gramineae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Pennisetum purpureum</i> , Schn., <i>Gramineae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Rottboellia cochinchinensis</i> Lour (Elayton), <i>Gr.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Sorghum arundinaceum</i> (Des. V) Stapf, <i>Gr.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Ludwigia abyssinica</i> A. Rich., <i>Onagraceae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Sida urens</i> L., <i>Malvaceae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Sphenoclea zeylanica</i> Gaertner., <i>Sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Cyperus difformis</i> L., <i>Cyperaceae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Fimbristylis littoralis</i> Grand, <i>Cyperaceae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Ageratum conyzoides</i> L., <i>Compositae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Eclipta prostrata</i> L., <i>Compositae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Physalis angulata</i> Linn., <i>Solanaceae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	

*: The variety of rice mostly attacked was Bouake 189; + Positive detection of RYMV infection; - Negative detection of RYMV infection; *Gr.* = *Gramineae*; *Sp.* = *Sphenocleaceae*

Table 2

Results of Enzyme-Linked Immunosorbent Assay (ELISA, A 405 nm) of leaf samples of rice and weeds collected from the upland rice scheme at Mbe, Cote d'Ivoire to detect rice yellow mottle virus infection in nature

Plant family and species	1996							1997						
	07	08	09	10	11	12	01	02	03	04	05	06	07	
<i>Gramineae</i>														
Rice (<i>Oryza sativa</i> L.)														
Indica type:														
Bouake 189	*	*	—	+	+	+	*	*	—	—	—	+	+	
BG 90-2	*	*	—	+	+	+	*	*	—	—	—	+	+	
Japonica type:														
WAB 56–50	*	*	*	—	—	—	*	*	—	—	—	—	—	
WAB 56–104	*	*	*	—	—	—	*	*	—	—	—	—	—	
ITA 257	*	*	*	—	—	—	*	*	—	—	—	—	—	
Moroberekan	*	*	*	—	—	—	*	*	—	—	—	—	—	
Weeds: <i>Gramineae</i>														
<i>Digitaria horizontalis</i> Willd.	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Eleusine indica</i> L.	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Panicum laxum</i> (L.) Sw.	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Imperata cylindrica</i> L.	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Cynodon dactylon</i> (L.) Pers.	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Rottboellia cochinchinensis</i> L.	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Sorghum bicolor</i> (L.) Dewitt	—	—	—	—	—	—	—	—	—	—	—	—	—	

*= Rice-free period; + Positive detection of RYMV infection; - Negative detection of RYMV infection

The consistent detection of RYMV in the rice samples is an indication that the virus subsists more on rice in the field. The infected ratooned/volunteer rice could serve as a source of inoculum for further infection to newly transplanted rice especially where fields are under cultivation throughout the year. Bakker (1974) and John et al. (1986) made similar observations. Volunteer crops have been found to perpetuate virus diseases from one season to the next (Zitter and Simons, 1980). The infection of the bait plants placed round the fields is an indication that a mobile vector was involved. In the lowland irrigation rice scheme at Sakassou the prevalent vector encountered in large numbers was *T. sericea*, which was suspected to be responsible for the transmission of RYMV to the bait plants and to the ratooned/volunteer lowland rice. In the upland rice ecology the vector encountered in large numbers was *Chaetocnema pulla* Chapuis, which was probably responsible for the transmission of RYMV on the bait plants. These insects are efficient vectors of RYMV (Bakker, 1974, 1975). Bakker (1974) and Fomba (1990) observed the fast spread of RYMV where high populations of *C. pulla* were found. A similar situation was observed in this study at Mbe, Cote d'Ivoire, where a highly susceptible lowland variety, Bouake 189, grown under irrigation, was severely attacked by RYMV. The upland rice varieties grown alongside it were not attacked. It is evident that RYMV subsists more on rice and that the lowland, indica type is more susceptible to the virus than the upland, japonica type. Therefore, the ratooned/volunteer rice that overwintered in the field could play a significant role in the survival of RYMV.

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Short communication

RESPONSE OF *TETRAGONOLOBUS PALAESTINUS* BOISS TO SEVERAL FREQUENCIES OF HAND WEEDING

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Field trials were conducted during the 1999 and 2000 growing seasons at Houfa, in northern Jordan, to study the effect of time of weed removal on the growth and yield of *Tetragonolobus palaestinus* Boiss. Removing weeds from 30 to 90 days after seeding (DAS) led to significantly larger yields than in plots which were not weeded. Maximum seed yield in both seasons was obtained when weeds were removed twice at 30 and 60 DAS.

Key words: legume, seed yield, weed competition

Introduction

Tetragonolobus palaestinus Boiss (belonging to the *Fabaceae* family) is a wild seed legume species which grows naturally on the plains and hilly areas of north Jordan and neighbouring countries (Al-Karaki, 2000). Jordanians consume the soft pods with immature seeds before they reach the dry stage. The search for new adapted legumes such as *Tetragonolobus palaestinus* Boiss will help to provide new food sources. The supply of protein to people living in this area could be enhanced by increasing supplies of food legumes rather than by organizing costly food programme based on protein-rich animal food (Al-Karaki, 2000). *Tetragonolobus palaestinus* Boiss is a very poor competitor and lack of adequate weed control may reduce yields by up to 40%. Simultaneous emergence and rapid growth of weeds lead to severe crop-weed competition, which culminates in heavy reductions in the growth and economic yield of the crop, thus lessening profitability (Yadav et al., 1983). Growers often assume, erroneously, that removing weeds at any time during the growing season is equally beneficial to the crop. However, substantial evidence in other legume crops (Yadav et al., 1984) indicates that the time of removal is as important as removal *per se*. The critical period is the time interval during which the crop should be free from weed interference to prevent losses (Radosevich et al., 1997). Therefore, weeds that emerge with the crop shortly after crop emergence continue to be a major problem in *Tetragonolobus palaestinus* Boiss production. The present study investigated the influence of weed removal at different times after seeding on the growth and yield of *Tetragonolobus palaestinus* Boiss.

Materials and methods

Field experiments were conducted at Houfa in northern Jordan during the rainy season of 1999 and 2000. The location has a Mediterranean climate (mild and rainy in winter, dry and hot in summer). A fertilizer treatment of $15 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$ was mixed with the soil prior to seeding. The experiments were laid out in a randomized complete block design (RCBD) with three replicates. The plots were $2.5 \times 2.4 \text{ m}$, of which $2 \times 1.5 \text{ m}$ was harvested to estimate yield. Each plot contained 6 rows with between and within row spacing of 0.4 m and 0.1 m, respectively, resulting in a planting density of about 25 plants m^{-2} . *Tetragonolobus palaestinus* Boiss was sown on 1 and 2 November in 1999 and 2000, respectively. The crop was harvested on 28 and 27 May, respectively.

In both seasons the experiment consisted of 7 treatments: an unweeded treatment (control), removal of weeds once at 30, 60 or 90 DAS, twice at 30 and 60 DAS, twice at 30 and 90 DAS and a weed-free treatment.

The intensity of weed incidence was recorded at 75% maturity using a quadrat of $50 \times 50 \text{ cm}$ placed on each plot. The weed control efficiency (WCE) was calculated using the following formula as reported by Singh et al. (2000):

$$\frac{\text{Dry matter of weeds in unweeded plot} - \text{Dry matter of weeds in treatment}}{\text{Dry matter of weeds in unweeded plot}} \times 100$$

Measured variables included pods plant^{-1} , seed weight plant^{-1} , number of branches, plant height (cm), number and weight of weeds m^{-2} . The weed count and dry matter of weeds were recorded per square metre and then, after drying in the oven at 70°C , the dry matter was converted to kg ha^{-1} . All data were statistically analysed by ANOVA and the treatment means were compared using least significant differences (LSD $P \leq 0.05$).

Results and discussion

Treatment effects on weeds

The weed flora of the experimental field consisted of *Hordeum murinum* L., *Vaccaria pyramidata* L., *Brassica nigra* L., *Convolvulus arvensis* L., *Moluccella laevis* L., *Galium tricornutum* L., *Anemone coronaria* L., *Cardaria draba* L., *Sinapis arvensis* L. and *Cuscuta* spp. The data given in Table 1 reveal that all the treatments significantly reduced the weed intensity and dry matter of weeds as compared to the unweeded control. Differences in weed number and weed dry weight were significant among the various treatments (Table 1).

Removing weeds once, at 30, 60 or 90 DAS, and twice, at 30 and 60 or 30 and 90 DAS, led to successively fewer being present at subsequent stages and subsequently fewer weeds at crop harvest than in the unweeded treatment (Table 1). The dry weight of weeds followed similar trends to the number of weeds. Weed removal at later stages of crop development gave the least dry weight of weeds at harvest since few additional weeds had time to emerge. The weed control efficiency ranged from 47.0 to 93.0%. The maximum weed control efficiency of 93.0% was recorded in the weed-free plots. Minimum weed control efficiency was recorded after weeding once at 30 DAS.

Table 1

Effect of time of weed removal on seed weight plant⁻¹ (SW, g), plant height (PH, cm), pods plant⁻¹ (PP), branches plant⁻¹ (BP), number (NW) and weight of weeds (WW, g) m⁻²

Treatments	SW	PH	PP	BP	NW	WW	WCE (%) [*]
Weed-free	12.0	48.5	28.5	11.5	10.5	6.0	93.0
Unweeded check	4.2	34.5	12.9	6.5	74.0	85.0	—
Weeding once at 30 DAS	9.0	39.5	23.5	9.5	28.0	43.5	47.0
Weeding once at 60 DAS	7.9	38.5	20.9	8.85	0.5	18.5	78.0
Weeding once at 90 DAS	4.2	35.5	12.9	6.5	11.5	6.5	93.0
Weeding twice at 30+60 DAS	12.5	47.5	26.0	11.0	28.5	10.5	88.0
Weeding twice at 30+90 DAS	11.5	39.5	23.5	9.5	31.0	26.5	69.0
LSD (0.05)	3.6	4.2	3.9	2.2	2.6	3.8	—

*: WCE = weed control efficiency; Data not analysed statistically.

Treatment effects on yield and yield components of Tetragonolobus palaestinus Boiss

Pods plant⁻¹, seed weight plant⁻¹ (g), branches plant⁻¹ and plant height (cm) were greatest in the clean-weeded treatment and least in the unweeded control (Table 2). If the weeds were not controlled until 90 DAS, they reduced the seed yield by 4.2 g plant⁻¹. Among the various treatments, the maximum seed yield of *Tetragonolobus palaestinus* Boiss was recorded when weeds were removed twice at 30 and 60 DAS. The increase in seed yield after weeding at 30 and 60 DAS was mainly due to the effective control of weeds, reducing the dry matter of weeds and the weed intensity, thus resulting in more pods per plant (Table 1) and finally higher seed yield.

The optimum time for weeding was twice at 30 and 60 DAS; the seed yield did not differ much from the clean-weeded treatment. Weeding once at 30 DAS yielded less than the weed-free plots and there was a steady decline in yield with later weeding.

In conclusion, weeds are one of the most important factors responsible for yield reduction in *Tetragonolobus palaestinus* Boiss. The critical period of crop-weed competition was from 30 to 60 DAS. Therefore, the treatments in which the weeds were removed twice at 30 and 60 DAS suffered less weed competition (for light, nutrients, moisture and space) during this critical crop period, which in turn resulted in better growth and yield components.

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Review

CHILLING TOLERANCE OF MAIZE

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The review will give a brief summary of the results achieved in the field of maize chilling tolerance in the Martonvásár institute in recent decades. The most important of these concern the joint effect of low temperature and light on photosynthetic processes, the role of light in the appearance of post-chilling symptoms, the importance of the alternative pathway of polyamine biosynthesis in the chilling tolerance of maize inbred lines, the confirmation of the correlation between glycine betaine and chilling tolerance, selection at the pollen level, and the role of certain protective compounds (salicylic acid and related compounds, S-methylmethionine and glutathione) in the enhancement of chilling tolerance.

Key words: chilling tolerance, chlorophyll fluorescence, glutathione, N-containing compounds, photoinhibition, pollen, salicylic acid, *Zea mays* L.

Introduction

As in many other countries, maize, which is of subtropical origin, is the most important fodder crop in Hungary. Its importance is proved by the fact that in recent decades the sowing area has moved ever further north in Europe. Hungary lies near the northern boundary of the grain maize production area and due to the continental climate, apart from summer drought, low temperature is the abiotic stress factor which threatens maize development and growth to the greatest extent, especially in the early growth stages. The production of hybrids with good chilling tolerance in the seedling stage is thus vital for reliable maize production. In an institute where maize breeding and crop production research has been underway for over 40 years, there is thus every justification for work on the theoretical and practical aspects of the low temperature effects to which the maize metabolism is exposed.

1. Breeding experiments

Chilling tolerance studies have always been an integral part of the maize breeding work in Martonvásár (Marton, 2000a; Szundy and Herczegh, 1986). In the earliest experiments Kovács (1961) found a close negative correlation between the duration of cold incubation and the germination percentage. Herczegh (1970) recommended the use of a new climatic programme (10 days' incubation at 8°C followed by 13.5°C) which made it possible to distinguish

more clearly between the chilling tolerance levels of the genotypes. Using this programme Szundy and Kovács (1981a, b) and Szundy and Marton (1999) found that a higher level of heterozygosity in the maternal parent had a favourable influence on chilling tolerance at emergence.

Experiments carried out by Marton (2000b) proved that the level of heterozygosity of the maternal parent also had an influence on chilling tolerance in the seedling stage. The results also indicated that chilling tolerance levels at emergence and in the seedling stage are subject to different genetic regulation, so selection must be carried out for both characters (Marton, 1990a).

Herczegh and Marton (1986) and Marton et al. (1990b) studied the heat minimum for the germination of hybrids and lines in the temperature gradient chamber. The temperature threshold values of the individual genotypes were found to differ considerably. In the suboptimal temperature range substantial differences could be demonstrated between the genotypes in the rate of germination (Marton, 1990b) and in the development of the young plants (Marton, 1991; 1997a; Marton et al., 1997; Marton and Szundy, 1997).

The results of chilling tolerance tests are significantly influenced by the pathogen infection of the germination medium and by the resistance of the genotypes (Marton et al., 1988a, b). Among the *Fusarium* species, infection with *Fusarium culmorum* (W. G. Smith) Sacc. and *Fusarium graminearum* Schwabe caused the greatest decline in chilling tolerance (Marton et al., 2000). The damage caused by fusarium seed infection increased as the temperature decreased (Marton et al., 1990a). As the result of fusarium seed infection there was a change in the role played by the genetic parameters responsible for the inheritance of chilling tolerance (Marton, 1997b).

The results show that chilling tolerance can be successfully improved by breeding (Herczegh, 1978; Marton et al., 1995). Under Hungarian conditions the production of high-yielding hybrids with longer vegetation periods, thus able to make better use of the ecological potential, is possible provided hybrids with better chilling tolerance are bred and sowing is carried out at an earlier date (Marton et al., 1999). The results suggest that earliness and chilling tolerance can be combined genetically in extra early hybrids adapted to conditions in Northern Europe (Pintér et al., 1995a, b).

2. Pollen studies

Research on pollen gained importance in tests on the effect exerted on progeny generations by maize pollen stored using the technique developed by Barnabás and Kovács (1988), and Barnabás et al. (1988), and in the elaboration of tissue culture methods. Both projects required the development of a sterile chilling tolerance test capable of adequately testing the effect of cold treatments without the soil and pathogen interactions involved in the traditional cold test. The chilling tolerance test elaborated by Bocsi (1988) proved suitable for studying the inheritance of chilling tolerance (Bocsi and Kovács, 1990; Bocsi et

al., 1990) and the influence of gibberellic acid treatments on chilling tolerance (Bocsi and Kovács, 1989). Tests on segregating generations demonstrated that chilling tolerance is a complex genetic trait determined by the effects and interactions of different gene complexes in various stages of plant development (Bocsi et al., 1990). The experiments also proved that the chilling tolerance of the gametophyte generation was closely correlated with the chilling tolerance of the seedlings (Kovács, 1989) and that this genetic overlapping between the vegetative and generative generations could provide a basis for the development of chilling-tolerant lines by means of pollen selection (Barnabás and Kovács, 1988; Kovács, 1989). The cold treatments applied during the storage process resulted, like pollen selection, in lines with significantly better chilling tolerance than that of hybrids produced using untreated pollen, demonstrating that genes responsible for chilling tolerance are expressed during the development and functioning of the pollen. The chilling tolerance of lines developed by repeated pollen selection was considerably greater than that of lines developed by means of traditional breeding (Kovács and Barnabás, 1992).

3. Characterisation of the chilling tolerance of young maize inbred lines using chlorophyll-a fluorescence induction parameters

The exposure of maize to suboptimal temperatures (below 13°C) during germination and early development had a negative influence on the photosynthetic apparatus of the plants. The F_o , F_v/F_m and F_R chlorophyll-a fluorescence induction parameters changed as the temperature dropped, indicating that several processes were responsible for the decreasing photochemical efficiency of photosynthesis. The synthesis of pigments and their incorporation into the photosynthetic membrane suffered damage, while Q_A reduction and the flow of electrons into the plastoquinone pool were inhibited. The 18 lines and 21 crosses examined could be ranked on the basis of chilling sensitivity using the values obtained for these indices at suboptimal temperatures, which were in good agreement with those obtained by breeders in various agronomic tests. On the basis of the results (Csapó et al., 1991; Kovács et al., 1992; Janda et al., 1994a, b; 1995; Páldi, 1986; 1990) it can be concluded that chlorophyll-a fluorescence induction is a reliable method for the practical evaluation of the chilling tolerance of inbred maize lines and crosses at suboptimal temperatures (Janda, 1998).

4. Effect of the photoinhibition occurring at low temperature

Earlier results (Janda et al., 1994a, b) showed that the higher the light intensity and the lower the temperature, the greater the changes induced in certain fluorescence induction parameters, including F_v/F_m . It was found that this latter parameter remained constant in the dark at 5°C even after 48 hours, while in the light ($200 \mu\text{mol m}^{-2} \text{s}^{-1}$) a substantial decline was observed after only a

few hours. It was also obvious from the results that the longer the plants were kept in the dark at low temperature, the more the efficiency of photosystem II (PSII) decreased when the plants were exposed to light. If cold treatment was carried out in the light, PSII was inhibited. Photoinhibition is important not only during low temperature stress, but also in the development of post-chilling symptoms (Janda et al., 1996; Szalai et al., 1996). There was also a drop in stomatal conductivity and transpiration. The net photosynthesis declined to an even greater extent in the light (Janda et al., 1997a; Szalai et al., 1997b). This could be attributed to the lower efficiency of the electron transport chain and the reduced activity of the enzymes responsible for carbon dioxide fixation, as confirmed by the high level of intercellular carbon dioxide (Janda et al., 1998).

5. Effect of low temperature on the etioplast structure and on chlorophyll biosynthesis

The studies were aimed at determining what correlation existed between the structure of maize chloroplasts and the effect of suboptimal temperatures (below 13°C). In order to make a detailed analysis of the Shibata shift (Shibata, 1957) the low temperature (77 K) spectra were measured after illumination for 5, 10, 20, 30, 40 or 50 minutes and the curves were split into their Gauss components: 674 and 690 nm for the emission spectrum and 672 and 684 nm for the excitation spectrum. The curves clearly indicated that the greatest difference in the Shibata shift occurred after approx. 40 min. illumination. An analysis of the low temperature fluorescence spectra of the leaves demonstrated differences between chilling-sensitive (Co 109) and tolerant (A 632) maize lines in suboptimum temperature treatments. These differences were not perceptible at 25°C. After illumination a delay in the Shibata shift could be registered in chilling-sensitive lines. This suggests a change in the geometrical parameters of energy transfer in cold-sensitive lines, associated with changes in the membrane structure during chilling treatment. Electron microscope studies proved that changes in the disintegration of the prolamellar bodies, preventing the translocation of the NADPH protochlorophyllide oxidoreductase enzyme, were correlated with chilling sensitivity and the temperature applied (Böddi et al., 1997).

6. Improvement in seedling chilling tolerance in maize using salicylic acid and its derivatives, or S-methylmethionine

Maize hybrids grown at normal temperature (22°C) suffered no visible damage during suboptimal temperature treatment (5 days, 2°C) in the presence of 0.5 mM salicylic acid (SA), while plants not treated with SA were destroyed (Janda et al., 1997b). Determinations of chlorophyll-a fluorescence parameters and measurements of ion leakage confirmed the protective effect of SA (Janda et al., 1999a, b; Szalai et al., 2000a, b). There was a significant increase in the

activity of guaiacol peroxidase and glutathione reductase, two enzymes involved in the antioxidant system. Compounds related to SA (acetyl salicylic acid, benzoic acid) exhibited a similar protective effect (Janda et al., 2000a). A new peroxidase isoenzyme band could be detected as the result of SA pre-treatment. The experiments proved that, as a member of the signal transduction chain, SA improves the chilling tolerance of maize through the induction of the guaiacol peroxidase and glutathione reductase enzymes (Janda et al., 1999a, b; Horváth et al., 2000).

Experiments carried out with the methyl derivative of methionine suggest that S-methyl methionine improves the osmotic potential and membrane stability of cells exposed to low temperature by stimulating the synthesis of polyamines, especially spermidine. This was confirmed by electrolyte leakage measurements (Rácz et al., 1998).

7. Effect of low temperature on the synthesis of certain N-containing compounds in inbred maize lines with different degrees of chilling tolerance

Maize lines with different degrees of chilling tolerance were exposed to various durations of cold treatment (1, 3, 5 and 8 days at 5°C) to study changes in the quantities of putrescine and agmatine, two major polyamines known to be excellent osmotic agents, and in those of proline and glycine betaine. Quantitative changes of varying extents were observed in these compounds in maize lines treated at low temperature. These changes were positively correlated with the chilling tolerance of the genotypes examined. The alternative metabolic pathway of polyamine biosynthesis, starting from arginine, which functions chiefly in higher plants and has agmatine as its key compound, plays a decisive role in the development of protective mechanisms against low temperature. The experiments also proved that glycine betaine, the quantity of which gradually decreased in chilling-sensitive lines as the result of chilling treatment but rose in tolerant genotypes, may be an important osmotic agent not only in drought and salt tolerance, but also in the defence against chilling damage (Döry et al., 1990; Páldi, 1995; Szalai et al., 1997a, b; Páldi et al., 1998; 2000a, b).

8. Role of glutathione in the chilling tolerance of young maize plants

Cold treatment, like other environmental stress effects, induces oxidative stress through the accumulation of reactive oxygen species. The antioxidant glutathione (GSH) plays an important role in the elimination of these reactive oxygen species (Kocsy, 1999; Kocsy et al., 2001a). In maize, chilling treatment increased the activity of several enzymes involved in GSH synthesis, thus leading to an accumulation of GSH (Brunner et al., 1995; Kocsy and Brunold, 1993; Kocsy et al., 1994). A comparison of maize lines with different degrees of chilling tolerance showed a greater chilling-induced increase in the activity of these enzymes and a faster accumulation of GSH in chilling-sensitive lines than

in chilling-tolerant ones (Kocsy et al., 1996; 1997a). The GSH level, the ratio of reduced and oxidised forms, the activity of the glutathione reductase regulating this ratio, and the chilling tolerance of maize could be manipulated successfully by chemical treatments (Kocsy et al., 1997b, c; 2000a, b; 2001b, c; von Ballmoos et al., 2000). The chilling tolerance of the chilling-sensitive maize line could be enhanced by stimulating GSH synthesis (Kocsy et al., 2001b) while that of the chilling-tolerant line could be reduced by inhibiting GSH synthesis (Kocsy et al., 2000a; von Ballmoos et al., 2000). The role of GSH in chilling tolerance is confirmed by the quantitative correlation discovered between the degree of chilling tolerance and the GSH level or the activity of glutathione reductase, which regenerates GSH (Kocsy et al., 2000a; 2001a).

9. Thermoluminescence experiments

The thermoluminescence (TL) emission of photosynthesising materials originates from the charge recombination between the positively charged donors and negatively charged acceptors of Photosystem II (PSII). Charge recombination between the S_2/S_3 states of the water splitting system and Q_B^- gives the B TL band peaking at around 30–35°C. The addition of diuron (electron transport inhibitor between the Q_A and Q_B quinone acceptors) leads via $S_2Q_A^-$ charge recombination to the Q TL band peaking at 5–12°C (Rutherford et al., 1982; Demeter and Vass, 1984).

Illumination of a long-term dark-adapted leaf or intact chloroplasts with far-red light induces an afterglow emission which can be optimally resolved as an AG TL band (Miranda and Ducruet, 1995). The AG band reflects a more complex phenomenon than the B and Q TL bands, which are specific for charge recombination within PSII. It is suggested that not only PSII, but also part of the cyclic electron pathway and the transthylakoid pH gradient are involved in the AG emission, which corresponds to a back electron transfer towards PSII centres initially in the S_2/S_3 Q_B state (Sundblad et al., 1988). The AG band may reflect the [ATP + NADPH] level in the chloroplast, as shown in *Mesembryanthemum crystallinum* L., a facultative crassulacean-acid-metabolism plant (Krieger et al., 1998).

It was shown that in control, unfrozen wheat plants, the T_{max} of the B band induced after 30 s far-red light at 0°C was approx. 15–18°C. In maize plants grown under the same conditions, this far-red-induced downshift was not so strong, since the B band peaked at 28–30°C (Janda et al., 1999c). There is usually a sudden drop in the AG band below a critical freezing temperature. However, while in wheat plants a weak TL emission could be seen in frozen samples between 40–50°C, in cold-sensitive maize plants this was completely suppressed and only the B band could be detected.

Chilling stress (0°C) in maize first caused a downshift and a temporary increase in the AG band after 4 h in the light, then a decrease in the AG and B TL bands after 1 day in the light. This decrease was less pronounced in cold-

tolerant genotypes and in those grown at acclimating temperatures (Janda et al., 2000b, c). The T_{\max} value of the AG band may also indicate the chilling tolerance of the maize genotypes (Ducruet et al., 1997a, b). After severe cold stress an additional band appeared above 80°C. This band indicates the presence of lipid peroxides (Vavilin and Ducruet, 1998).

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Review

STRATEGY FOR MANAGING NATURAL RESOURCES IN FLOOD-PRONE ECO-SYSTEMS FOR SUSTAINABLE AGRICULTURAL PRODUCTION

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Flood-prone environments receive 50 to 400 cm water for more than a month during the wet season, depending on the amount and duration of rainfall, and the depth, time and frequency of flooding. In India, 2.5 million ha flood-prone areas are to be found, mostly in the eastern states of Assam, West Bengal, Uttar Pradesh and Bihar. No crops other than rice can be grown on these lands; however, rice has very poor productivity (1.0–1.5 t/ha) due to the unpredictable growing environment.

There are three options to develop better production technologies for this harsh ecology: (i) rice mixed cropping, (ii) winter rice cultivation, and (iii) rice + fish farming. Each one has its merits and demerits. Adoptable technologies have been developed which have been verified in the field. Integrated farming including rice + pulses + vegetables + green manuring + fish has also been found successful with some modifications in the field. Experiments both at research stations and on farmers' fields have shown that the disadvantageous, harsh ecology of fragile flood-prone environments could be made as productive as other ecosystems through the use of adoptable technologies.

Key words: flood-prone ecology, environment, rice cropping, production strategy, integrated farming system

Introduction

Flood-prone environments are characterized by a great diversity of growing conditions, depending on the amount and duration of rainfall, the depth and duration of flooding, the frequency and time of flooding, etc. In most Asian flood-prone areas, flooding during the wet season occurs from June to November with the maximum water depth from August to September and a recession of water from October onward. Most of the flood-prone areas in India (2.5 million ha) are found in the eastern states of Assam, West Bengal, Bihar and Uttar Pradesh (Catling, 1984). In these agricultural lands no crops other than rice can be grown in the wet season. Productivity is, however, poor mainly due to the unpredictable growing conditions. There are two main options to increase the productivity of this eco-system:

(i) To devise effective flood-control measures and develop better water management practices, or

(ii) To develop a suitable technology for higher productivity without changing the existing crop growing conditions.

The first option would require enormous investments and keeping in mind the economic conditions in the states concerned, many years would be required for its accomplishment. The second option, therefore, has relevance particularly for agricultural scientists to design and develop a sustainable technology for this adverse, fragile, flood-prone ecology in order to provide food security for the millions of resource-poor farmers whose economic well-being depends on the harvest.

Bihar State lies in the Indo-Gangetic belt of Eastern India and is the 6th largest rice-growing region of the world. It has diverse ecological situations, ranging from upland to very deepwater conditions, and consequently vast areas in north and south Bihar are flood-prone or flood-affected (Fig. 1). The flooding pattern not only differs from region to region but even from locality to locality. There are thus no agricultural technologies which are universally adaptable for the whole region. An example of a case study made by rice scientists in Eastern India may be cited to elucidate the point. A cluster of four representative villages near the embankment of the Burhi Gandak River in north Bihar was subjected to agro-ecological analysis (Loghtfoot et al., 1991). Four types of lands were categorized, namely (i) upland, (ii) midland, (iii) lowland and (iv) deepwater on the basis of water depth and period of stagnation during the wet season. This is primarily due to the contour of the land, and is generally valid for most of the area. Upland and midland constituted 15–20% of the total cultivable area, while the rest was flood-prone. The flooding pattern in a typical saucer-shaped depression in the Biraul region of eastern India is shown in Figure 2.

Keeping in mind the typical problem of this region, an attempt has been made to summarize the results of the painstaking work undertaken over a number of years to tame this flood-prone ecology of Bihar to achieve optimum productivity. The first step was to characterize the growing conditions and then to generate adaptable technologies. The results have far-reaching implications and were tested in the field.

Flood-Prone Ecologies of the Bihar Plains

Physiographically, the Bihar Plains can be divided into two distinct regions:

- (i) The North Bihar Alluvial Plains, and
- (ii) The South Bihar Alluvial Plains.

North Bihar Alluvial Plains

The land in this region is particularly flat with its slope towards the southeast. Many natural rivers, namely the Gandak, Ghaghra, Burhi Gandak, Kamala Balan, Bagmati, Kosi Balan, Mahananda and their tributaries, serve as the natural drainage for the plain. The region has two distinct agro-climatic zones: northwest and northeast.

The northwest plain (Zone I) covers the Saran and Tirhut divisions of Bihar. The zone slopes towards the southeast as is evident from the direction of the rivers. However, all the rivers finally merge in the River Ganges. As a result a vast water-logged area has developed in the districts of Saran, Vaishali, Samastipur, Muzaffarpur and Begusarai. Due to the near flatness of the landscape and the saucer-shaped depressions between the rivers, vast areas in these districts are flooded during the rainy season. When the floodwater recedes from other areas, the saucer-shaped depressions and the abandoned channels of rivers, lakes, etc. remain flooded for various lengths of time. Due to the construction of irrigation structures and canal systems as part of the Gandak Irrigation Command project, many natural drainage channels have been disturbed, causing more flooding and water logging in the central and western part of the zone. Embankments and roads have also aided water-logging. The River Gandak serves as a grand drainage channel in which all the rivers meet at various points.

The northeast plain (Zone II) covers the Darbangha and Kosi divisions of Bihar and has a general slope towards the southeast. The Kosi, the Mahananda and the Ganges are the major rivers. This zone is full of abandoned beds and dead channels of the Kosi River and its tributaries; small lakes or marshy grounds are also frequently encountered. There are vast tracts of waterlogged areas in the north Bihar plains, which remain inundated for a considerable period (July to December) with a water depth of as much as 3 to 4 metres.

South Bihar Alluvial Plains

The plain has a general slope towards the north-east. Important rivers other than the Ganges are the Sone, Punpun, Falgu, Badua, Harohar, Kiul, Karmnasa and Chir. Except the River Sone, all are seasonal rivers. The region has comparatively better irrigation resources (68% irrigated area) and the lands have normal plain physiography, unlike the north Bihar plains (42% irrigated area) that have upland to deep lowland physiography. Rice and wheat are the major crops.

Tal and *Diara* are two types of typical land conditions found in this zone. *Tal* is a vast stretch of bowl-shaped land lying south of the natural levee of the River Ganges, spread over about 0.1 million ha, which remains inundated during the rainy season. Winter crops, mostly pulses and oil seeds, are cultivated under rainfed conditions after the recession of the floods. The soils are generally heavy in texture and crack heavily on drying, leading to rapid moisture depletion. *Diara* lands are a flood-prone area formed due to the meandering and course changing of perennial rivers. They are generally found on both banks of the river but may also be formed in between two courses of the same river as an island. This land becomes inundated for different periods in the rainy season when the rivers are in spate and is prone to periodical erosion. Such areas cover 0.11 million ha and have a light texture. Wheat, maize, mustard and pulses are grown as winter crops. During the summer cucurbits are the main crops.

Water stagnation and crop management for the North and South Bihar Alluvial Plains

The North and South Bihar plains may be classified in nine categories based on the period of water stagnation and consequently the water depth (Fig. 3). They are primarily rice lands. About 2.7 million ha rainfed lowlands in various categories remain submerged for considerable periods. The longer the duration of stagnation, the greater the depth of the water. Rice yields are poor and no other crop could be cultivated in place of rice. As the period of stagnation in midlands is shorter, subsequent winter crops can be grown. However, management is needed for the lowlands and deepwater areas. Due to the longer period of water stagnation, the subsequent winter crop is often delayed. The low-lying areas of North Bihar are connected with each other. Saucer-shaped land depressions or abandoned river courses have gradations in their water depth patterns (Fig. 4). The deeper parts remain waterlogged for a longer period, and could be used as a source of irrigation for the second crop. Moreover, these areas are close to rivers, where water is plentiful and could again be used as an irrigation source.

Features of Flood-Prone Lands

Water stagnates for considerable periods (ranging from 4 to 12 months) on an area of approximately 0.7 million hectares, while 0.65 million ha are under deep water (water depth varies from 0.5 to 4 m) and 0.05 million ha are subject to flash floods. Abandoned riverbeds are also found on the North Bihar Plains. They are scattered throughout the region (Fig. 5) and vary in shape, size and depth. The saucer-shaped depressions are deeper in the centre and shallower at the periphery, while the former riverbeds form long strips (Fig. 6) and many are found in chains. These are vast natural water resources, which are virtually untapped. This is one of the important themes of the present paper, which is discussed hereafter on a practical footing based on the results of experiments conducted both at research stations and on farmers' fields.

Options for Increasing Productivity in Flood-Prone Land

There are three options for increasing the productivity of this fragile flood-prone ecosystem, namely:

- (i) Rice in a mixed cropping system.
- (ii) Winter rice and integrated farming system. Summer rice is possible where irrigation water is available.
- (iii) An integrated farming system is possible where the water depth varies from 1.0–1.5 m.

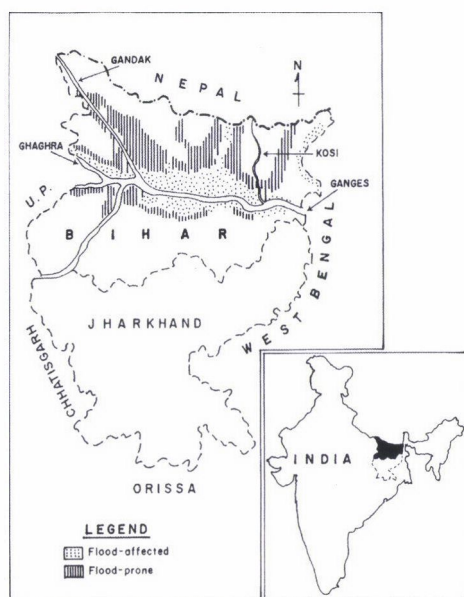


Fig. 1. Flood-prone and flood-affected areas of Bihar

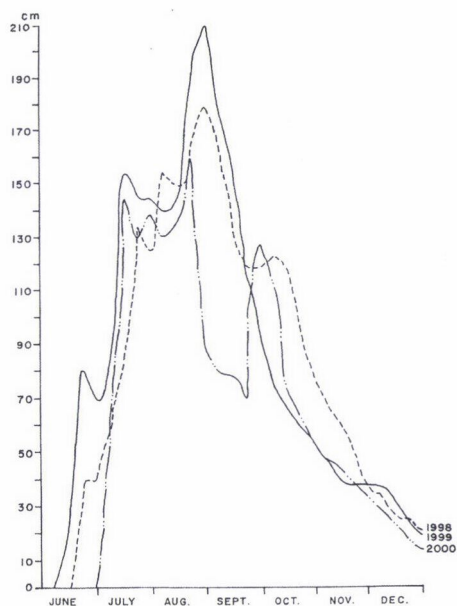


Fig. 2. Flooding pattern Darbhanga district

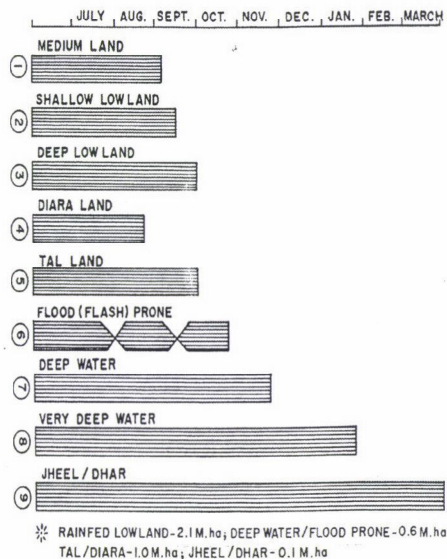


Fig. 3. Rainfed lowland rice and period of stagnation

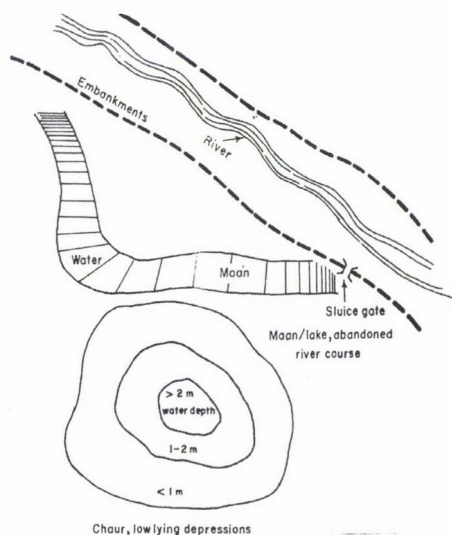


Fig. 4. Types of deepwater areas in Bihar

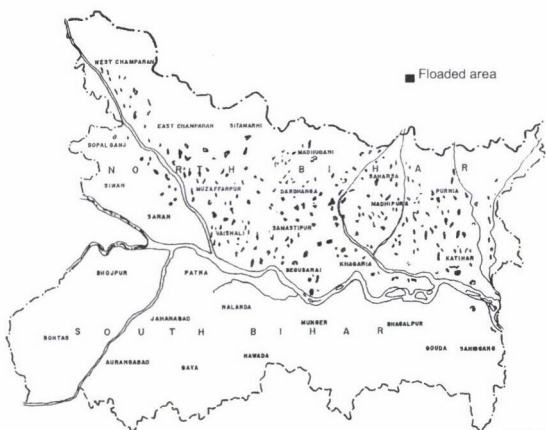


Fig. 5. Flood-affected area in Bihar plain

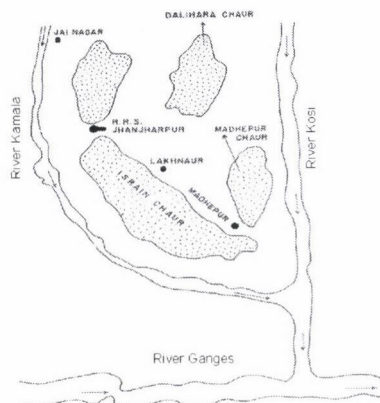


Fig. 6. A view of land area with chauras (depressions) between Kamala and Kosi rivers

(i) Rice in a mixed cropping system

The rice crop is usually broadcast mixed with mungbean, sesame, fodder, sorghum, jute, etc. in summer (Thakur et al., 1984). Mixed cropping induces a fair degree of sustainability in the system. The mixed crops are harvested before the floods in June–July. When the floods are severe, the rice crop may also fail. Extensive research on mixed cropping was conducted on farmers' fields by a Ford Foundation project on "Farming Systems Research & Extension" over a number of years. Types of rice varieties, the mixed crop concerned and the seed rate proportion were investigated. Total yield was calculated in terms of rice yield equivalence. Yields as high as 4 t/ha were achieved with rice + mungbean (pulse) in the Tirhut region of Bihar and rice + jute in the Kosi command area of Bihar, where investments were virtually nil (Thakur et al., 1994). Earlier Singh et al. (1998) suggested suitable cropping patterns based on rainfall and water depth pattern.

In normal years, the water in the peripheral portion of the depressions dries up in November–December, while in the central portion the water remains up to March. However, the situation varies yearly due to variations in rain and floods. Singh et al. (1998) suggested various cropping patterns for the changing environment. Thakur (1989) devised better means of exploiting the water resources in the depressions for rice cultivation and visualized a rice revolution in Bihar. Siddiq (1997) suggested expanding winter rice cultivation on nearly one million hectares of waterlogged area in Bihar. Since its productivity is very high and assured, it is popular in the Kosi range adjoining West Bengal State, where rice became very popular with the advent of high-yielding varieties. Its cultivation did not spread beyond the Kosi range, the major impediment being

tolerant varieties will be required. Recently a variety, Gautam, has been released which has very high yield potential (as high as 9 t/ha) due to a high degree of cold tolerance at the seedling stage (Thakur et al., 1994a). This variety has set a trend in yield and has become very popular in a short period.

(ii) Winter rice cultivation

High-yielding dwarf varieties of rice have become popular in the winter season in West Bengal. This technology has spread to the adjoining districts of Katihar, Purnea and Saharsa. Nearly 0.1 million ha are now planted to winter rice, mostly replacing the deepwater depression lands (Thakur et al., 1990). The yield ranges from 3 to 5 t/ha. Though this region is congenial in respect of groundwater and low temperature severity, low temperature invariably damages seedlings in the nursery. For the expansion of winter rice cultivation in other regions, cold-tolerant varieties at the seedling stage will be required. Popular varieties like IR 8, Pusa 2-21, Saket-4, etc., though popular in the winter season, cannot tolerate temperatures below 10°C. In winter rice variety trials, a mutant of Rasi, PSRM 1-16-4B-11, performed exceedingly well in the winter season, with an average yield of 7.5 t/ha over a number of years. This was later released as Gautam (Thakur et al., 1990). It has also yielded 7.0 to 8.8 t/ha in other districts. A greenhouse trial is now being conducted at the Land, Water, Environment and Engineering Research Programme, Patna to evaluate the seedling emergence of winter rice at cold temperatures below 10°C (Singh and Singh, 2001).

(a) Possible area for winter rice cultivation

The depressions found all over north Bihar are suitable for winter rice cultivation. Irrigation could be possible either from the water available in the depression or from tube wells. Besides this, the waterlogged and marshy lands near roads and embankments, where no crops are grown, would be ideal areas for winter rice cultivation. The area around the Sone canals remains waterlogged for a long period and virtually remains fallow. Thus nearly 1 million ha of land in Bihar are potential areas for winter rice cultivation. The total yield of this area, if it were sown to winter rice, would be almost equal to the total yield of wet rice, grown on about 5 million ha. It is essential to develop a number of suitable winter varieties to meet the growing requirements. It is not visualized to replace wheat in the winter season, but to grow winter rice in areas where wheat is not possible due to waterlogging, like the Madhubani district. The yield of winter rice has been found to be 6.4–9.0 t/ha (Thakur et al., 1997).

(b) Integration of winter rice with deepwater cultivation

Usually, winter rice is mono-cropped and requires a high input for higher yield. With the advent of high-yielding varieties, a major area of deepwater land in Bangladesh shifted to winter rice when irrigation resources were available.

However, there is a trend to integrate winter rice with deepwater rice (Catling et al., 1983). This was investigated at Biraul in a typical saucer-shaped depression. Deepwater rice was broadcast in the standing winter rice crop and after the winter rice harvest, proper weeding and top dressing with 20 kg N/ha were carried out. In spite of some water availability problems, the winter rice yielded 3.5 t/ha, whereas the deepwater rice yielded 1.4 t/ha.

(iii) *Integrated farming system (rice + fish farming)*

In deepwater rice fields, fish can be raised without effort. Their turnover is, however, poor. Studies at the Rice Research Station, Chinsurah, West Bengal showed the possibility of culturing fish with rice with remarkable turnover using a supplementary fish feed treatment (Thakur, 1989). This was also investigated at the Rajendra Agricultural University farm, Pusa in deepwater rice experiments where the soil of the peripheral portion of land was dug out to a depth of 1.0–1.5 m and 2 m across with remarkable success. The fish were collected after the rice harvest in late November. In normal depressions the fish leave when the water recedes, while in these trenches they remain for a longer period.

An experiment on this integrated farming system was carried out at the State Agricultural University farm, Pusa (Bihar) during 1993–94 and 1994–95 on 0.4 hectare land in a deepwater ecosystem. A trench of 1 m width was dug out around the field and the soil was used to raise the dike about 1 m high. The improved photosensitive tall rice variety *Vaidehi* was direct seeded at 70 kg/ha mixed with mungbean (*Vigna radita* L.) var. NP18 at 9.0 kg/ha on March 8, 1993 and March 5, 1994. A basal dose of 20 kg N per hectare was applied. The mungbean was harvested 70 days after sowing (early June) before the onset of the monsoon. The rice was hand-weeded once after the mungbean was harvested. The fuel crop dhaincha (*Sesbania rostrata*) was sown in mid-June on the inner side of the dike around the field. The standing crop of rice was top dressed with urea at 20 kg/ha after the first rain. In the month of July, locally adapted fingerlings were added at a rate of 7500/ha to the submerged rice field. Cow dung at 50 kg and oil cake at 4 kg/week were used as fish feed. Climbing vegetables (*Luffa* spp.) were sown on the top of the dike in July, while winter pigeon pea (*Cajanus cajan* var. Navin) was sown on the outer side of the dike by 15th September at 60 kg seed/ha, after which 100 kg/ha DAP was also applied to the crop. The fish remained in the main field up to September and in the trenches up to their harvest in mid-November. Rice and *Sesbania* (for seed and fuel) were harvested in early and late December, respectively. The vegetables grew well on the dike and as many as 5 pickings were taken. The harvesting of pigeon pea was done by mid-April. The comparative economics, calculated at the local market rate, showed that the adoption of the integrated approach resulted in up to 88.7% higher net returns in comparison to traditional practices (U.S.\$ 240.68/ha year). In other words by investing an additional U.S.\$ 94.31/ha in the integrated farming system, an additional income of U.S.\$ 213.94/ha could be obtained (Singh and Singh, 1998).

It can be concluded that the integrated approach would be beneficial to the farming community in many ways, as the basic requirements of a household, like food grains, vegetables, fish and fuel, can be met very well from the limited area and will also provide employment during the off-period.

Strategies for the Future

Low-lying areas of north Bihar are subjected to uncontrolled flooding and considered unproductive, but the area is amenable to higher production. Rice in a mixed cropping system, winter rice, and rice + fish farming are suitable options, each of which has its own importance. A project involving experts on agriculture, fisheries, irrigation/drainage and flood control is needed to expand the research and utilize the vast water resources. To tackle the problem of the entire region is complex, but is feasible if executed stepwise with linkage of all concerned on a regional basis. Many agencies are working in the region. Linkage among them will make efforts successful for an integrated farming system.

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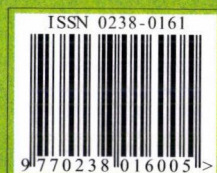
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EFFECT OF CONDITIONING APPLE SHOOTS WITH META-TOPOLIN ON THE MORPHOGENIC ACTIVITY OF *IN VITRO* LEAVES

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The effects of a new type of aromatic cytokinin, meta-topolin, on the morphology and histology of apple leaves and its post-effects on the subsequent shoot regeneration from *in vitro* leaves were studied in cv. Royal Gala. The media applied for pre-treatment differed from each other in their cytokinin composition: medium No. 1 contained no cytokinin, No. 2 was supplemented with 0.5 mg l⁻¹ benzyladenine, while Nos. 3–6 contained meta-topolin, the new type of cytokinin, in four concentrations (0.5–1.0–1.5–2.0 mg l⁻¹). After a 3-week pre-treatment on these media shoot regeneration was induced on two test regeneration media containing thidiazuron (0.2 mg l⁻¹) or benzyladenine (5.0 mg l⁻¹). Irrespective of the pre-treatments, high regeneration (97–100%) was observed on all the regeneration media. However, the conditioning of apple shoots for three weeks on medium supplemented with meta-topolin in a concentration range between 0.5 and 1.5 mg l⁻¹ caused a significant decrease in the rate of vitrified shoots (down to 13.4%) and increased the number of regenerated shoots per leaf segment significantly (up to 15.1). There was a positive correlation between the histological status and regeneration capacity of *in vitro* leaves. According to these results, meta-topolin, as a new source of cytokinin, could increase the morphogenic potential of apple leaves.

Key words: meta-topolin, conditioning, anatomical structure, organogenetic index

Introduction

Regeneration from somatic tissues, such as leaves, is a key process in improving tree species by genetic manipulation or identifying somaclonal variants (James and Dandekar, 1991; Korban et al., 1992). Numerous studies on the regeneration of apple have been published and organogenesis has proved to be influenced by several factors, such as age and source of explant, hormonal balance of the regeneration medium, culture conditions, type of vessel, etc. (James et al., 1988; Welander, 1988; Fasolo et al., 1989; Predieri and Fasolo, 1989; Dufour, 1990; Standardi and Houshmand, 1992; Korban et al., 1992; Famiani et al., 1994; Yepes and Aldwinckle, 1994; Ferradini et al., 1996). In general, good regeneration could be obtained in several apple genotypes using MS basal medium (Murashige and Skoog, 1962) supplemented with benzyladenine (BA) or thidiazuron (TDZ) as cytokinin and naphthalene acetic

acid (NAA) as auxin (Sriskandarajah et al., 1990; Swartz et al., 1990; Korban et al., 1992; Famiani et al., 1994; Yao et al., 1995). However, several undesirable side effects of TDZ, such as vitrification, forming of fasciated shoots, etc., were observed, especially at concentrations higher than 1 μM (0.2 mg l^{-1}) (Huetteman and Preece, 1993).

The effects of various factors influencing organogenesis were studied mostly during the regeneration phase, and there are few reports considering the post-effects of culture conditions prior to the regeneration phase (McHughen et al., 1989; Swartz et al., 1990; Ferradini et al., 1996; Sriskandarajah and Goodwin, 1998).

Meta-topolin (TOP) was reported to be a highly active aromatic cytokinin (Kubaláková and Strnad, 1992; Werbrouck et al., 1996; Strnad et al., 1997) in tissue culture of other plant species, such as *Spathyphyllum* and *Beta vulgaris*. Previously, TOP was found, to improve the physiological status of leaves in apple shoot culture (Dobránszki et al., 2000), so its conditioning effect was studied on leaves prior to regeneration. The aim of this work was to study the effects of a newly isolated cytokinin, meta-topolin (Horgan et al., 1975), in different concentrations on the organogenetic ability of apple leaves in the last subculture prior to regeneration compared with the effects of a last subculture on medium with BA or without cytokinin.

Materials and methods

Plant material

In vitro shoot cultures of apple (*Malus × domestica* Borkh.) cultivar Royal Gala (=Tenroy) were used in the experiments. Shoot explants were placed horizontally on shoot proliferation medium consisting of MS (Murashige and Skoog, 1962) basal medium supplemented with 100 mg l^{-1} myo-inositol, 0.7 % agar-agar, 3 % sucrose, 1.0 mg l^{-1} 6-benzyladenine riboside (BAR), 0.3 mg l^{-1} indole-3-butyric acid (IBA) and 0.2 mg l^{-1} gibberellic acid (GA_3). The pH of the medium was adjusted to 5.8 before autoclaving and the medium was autoclaved for 20 min at 121°C and 10^5 Pa in an ST-124/2 autoclave. The cultures were grown at 22°C with a 16 h photoperiod provided by warm-white lamps (Tungsram F74) at a PPFD of 105 $\mu\text{mol s}^{-1}\text{m}^{-2}$. The shoots were subcultured and multiplied at 4-week intervals to obtain sufficient shoots for regeneration experiments.

Pre-treatments of shoots

The effects of 3-week shoot pre-treatments were studied on the potential for regeneration. In the pre-treatments 3-week-old shoots having 5–7 leaves with a length of about 35–40 mm were placed on pre-treating media consisting of Murashige and Skoog (1962) basal medium supplemented with the components used for shoot proliferation. Only the cytokinin supply was different. The pre-treating media contained two different types of cytokinins in the following concentrations: No. 1: no cytokinin was added to the medium; No. 2: 0.5 mg l^{-1} BA; No. 3: 0.5, No. 4: 1.0, No. 5: 1.5 and No. 6: 2.0 mg l^{-1} meta-topolin (TOP) were added to the media. The pre-treatments were carried out in Kilner jars (400 ml, 75 mm inside diameter and 85 mm long) and five shoots were placed vertically on 40 ml of medium in each jar. The cultures were grown under the same environmental conditions as used during shoot multiplication.

At the end of the pre-treatments the morphology and histology of the upper two leaves were examined. The length and width of the leaves were measured and the leaf surface (length × width) was estimated. Measurements were made from 25 jars in each treatment and the experiment was repeated twice.

For the histological studies the 1st and 2nd leaves, also used for regeneration, were collected from pre-treated shoots. Leaf samples for light microscopy were fixed in 5% glutaraldehyde for 2 hours followed by 1.5-hour treatment with osmium tetroxide and dehydration in a graded acetone series. After dehydration the samples were embedded in Spurr resin and stained with toluidin blue. Cross-sections were made from the leaves using an ultramicrotome (Reichert).

Regeneration from leaves

After a three-week pre-treatment the upper two, fully expanded young leaves were used for regeneration. The petiole and apex of the leaves were removed and the leaves were cut transversely into two strips (about 5 mm wide). All cuts were made in a solution of citric acid (0.15 g l^{-1}) and ascorbic acid (0.1 g l^{-1}). The leaf explants were then placed with the adaxial side down on two regeneration media (36 explants per combination of pre-treatment and regeneration). The regeneration media consisted of Murashige and Skoog salts, B₅ vitamins supplemented with 100 mg l^{-1} myo-inositol, 0.25% gelrite, 3% sucrose, 0.2 mg l^{-1} NAA and either 0.2 mg l^{-1} TDZ (R1) or 5.0 mg l^{-1} BA (R5). The explants were incubated in the dark at 24.5°C for 3 weeks, then in the light at 22°C with a 16 h photoperiod for another 4 weeks. The light intensity was increased weekly, from $35 \mu\text{Mol s}^{-1} \text{ m}^{-2}$ during the first week to $70 \mu\text{Mol s}^{-1} \text{ m}^{-2}$ during the second week and $105 \mu\text{Mol s}^{-1} \text{ m}^{-2}$ from the third week.

Data analysis

After seven weeks the number of explants with regenerated shoots (=regeneration percentage), the number of regenerated shoots per explant, and the number of explants with vitrified shoots (=vitrification percentage) were recorded. The data were analysed statistically by one- and two-way ANOVA followed by Tukey's test using SPSS 7.5 for Windows software. The data, given as percentages, were non-parametric, so they were analysed after arcsine transformation. The results are presented in non-transformed format. The experiments were repeated twice. The organogenetic index was calculated from the data as follows:

$$\text{OI} = (\text{regeneration percentage} - \text{vitrification percentage}) \times \text{shoot number per organogenetic explant} / 100,$$

where OI = Organogenetic index

This calculation, based on the organogenetic index used by Famiani et al. (1994), excluded the shoot length, but the index included the vitrification percentage because of its great importance in shoot regeneration.

Results

Effects of pre-treatments on leaf morphology and histology

After 3-week pre-treatments studies were made on the morphology (leaf size) and histology of the upper two well-expanded leaves, which were used for regeneration. The leaf surfaces, estimated from the width and length of the leaves, were influenced by the pre-treatments (Table 1). The leaf area of both the 1st and 2nd leaves responded to the cytokinin content of the medium in the same way. The highest leaf area was achieved on medium containing 0.5 mg l^{-1} TOP (89.8 mm^2 in the case of the 1st leaf and 79.6 mm^2 in the case of the 2nd leaf). The leaf area decreased significantly (from 89.8 to 70.9 mm^2 and from 79.6 to 56.3 mm^2 , respectively) when the TOP concentration was increased from 0.5 to 1.0 mg l^{-1} . However, a further increase in the TOP concentration up to 2 mg l^{-1} did not cause any further decrease in the leaf surfaces. The leaf surface on shoots grown on cytokinin-free medium did not differ significantly from that obtained on media with higher concentrations of TOP. The smallest leaves developed on medium containing 0.5 mg l^{-1} BA as cytokinin.

Table 1
Effect of pre-treatments on leaf area of 1st and 2nd leaves after culturing shoots for 3 weeks*

Pre-treatments	Leaf area (mm ²) of	
	1 st leaf	2 nd leaf
No. 1: cytokinin-free	63.7b	59.0b
No. 2: 0.5 mg l ⁻¹ BA	47.8a	34.8a
No. 3: 0.5 mg l ⁻¹ TOP	89.8c	79.6c
No. 4: 1.0 mg l ⁻¹ TOP	70.9b	56.3b
No. 5: 1.5 mg l ⁻¹ TOP	74.4b	57.9b
No. 6: 2.0 mg l ⁻¹ TOP	68.6b	59.6b

* Small letters in the columns indicate homogeneous groups (P<0.05)

The anatomical structure of the 1st and 2nd leaves of shoots grown on the same pre-treating medium showed no differences, but there were great differences in the anatomical structures of leaves originating from the different pre-treating media (Fig. 1). The cross-section of leaves harvested from the cytokinin-free medium showed a well-differentiated structure (Fig. 1A). The epidermis consisted of a single cell layer. A high number of closed stomata was found on the abaxial epidermis. The spongy parenchyma contained very large intercellular spaces. Its structure was very similar to the structure of *in vivo* leaves described in other woody plants (Kiss et al., 1999) but the cell walls were thinner. The vascular bundle was also well developed.

Leaves from BA-containing medium showed a structure like that of mature *in vitro* leaves (Kiss et al., 1999; Jámbor-Benczúr et al., 2001) (Fig. 1B). The adaxial and abaxial epidermis consisted of one cell layer each and differed from each other. There were stomata on the abaxial epidermis which were mostly opened and often protuded above the surface of the epidermis. One row of elongated palisade cells could be observed. The intercellular spaces in the spongy parenchyma were much smaller than after cytokinin-free pre-treatment (Fig. 1A).

The transverse section of the 1st and 2nd leaves from shoots pre-treated with TOP in a concentration range of 0.5 to 1.5 mg l⁻¹ showed a structure like that of immature *in vitro* leaves (Fig. 1C–E). This type of structure can be characterized by very few immature and opened stomata on the abaxial epidermis, the compact structure of the palisade and spongy parenchyma, parenchyma cells with dense cytoplasm and an immature vascular bundle. After pre-treatments with 0.5 mg l⁻¹ TOP, two rows of palisade cells could still be observed (Fig. 1C). However, when the TOP concentration was increased to 1.5 mg l⁻¹, the mesophyll was homogeneous, and the palisade and spongy mesophyll layers were hardly distinguishable (Figs. 1D and 1E). When the concentration of TOP was increased to 2 mg l⁻¹, a more differentiated leaf structure could be observed: the palisade and spongy parenchyma cells were distinguishable and two rows of palisade cells were detected (Fig. 1F).

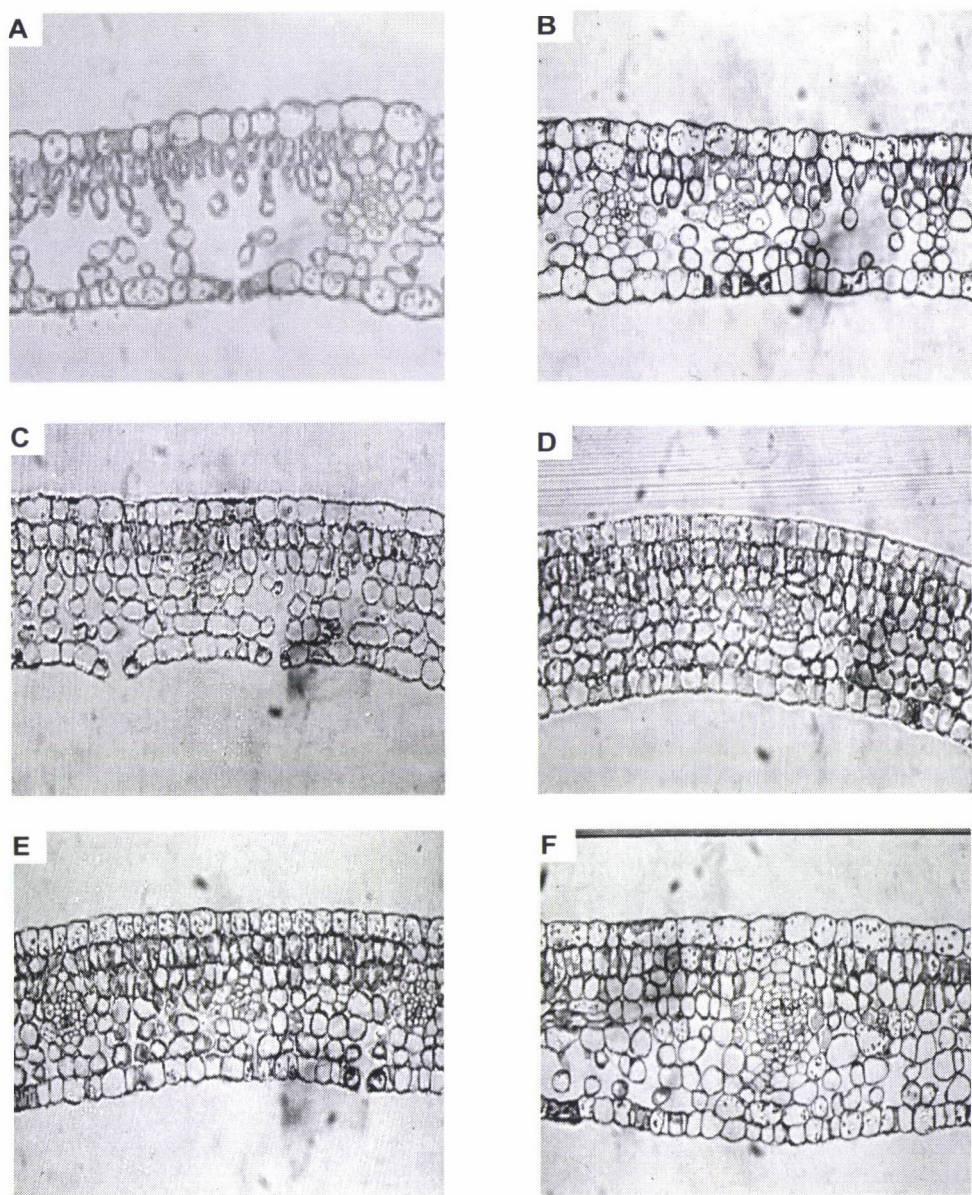


Fig. 1. Light micrographs of 1st leaves from control (A, B) and conditioned (C, D, E, F) shoots. Cross-sections of leaves after pre-treatment on cytokinin-free (A) and BA-containing (B) media. Leaf structure after pre-treatment with 0.5 (C), 1.0 (D), 1.5 (E) and 2.0 mg l⁻¹ (F) TOP $\times 40$ (Detailed description in the text.)

Post-effects of cytokinins on regeneration from leaves

After the pre-treatments, shoot regeneration was induced on two test regeneration media containing 0.2 mg l⁻¹ TDZ (Fasolo et al., 1989) or 5.0 mg l⁻¹ BA (Welander, 1988; Yao et al., 1995). Neither the pre-treatments nor the regeneration media influenced the regeneration percentage significantly. High regeneration (97–100%) was observed in all the treatment combinations, but the pre-treatments caused great differences in the vitrification percentage, which ranged from 36.2% to 100% on regeneration medium containing TDZ and from 13.4% to 56.8% on regeneration medium containing BA (Table 2).

The conditioning of apple shoots on media containing TOP before regeneration is able to decrease the rate of vitrification (Table 2) and increase the number of regenerated shoots (Table 3) compared with pre-treatments on media free of cytokinins or containing BA.

Table 2
Effects of pre-treatments and regeneration medium on the percentage of vitrified shoots*

Pre-treatments	Main effect of pre-treatment: % of vitrified shoots	Effect of regeneration medium: % of vitrified shoots	
		R1	R5
No. 1: cytokinin-free	74.3c	88.8c, B	56.8c, A
No. 2: 0.5 mg l ⁻¹ BA	66.7c	100.0c, B	26.8b, A
No. 3: 0.5 mg l ⁻¹ TOP	42.4a	66.5ab, B	13.4a, A
No. 4: 1.0 mg l ⁻¹ TOP	37.5a	47.2a, B	27.8b, A
No. 5: 1.5 mg l ⁻¹ TOP	34.8a	36.2a, A	33.2b, A
No. 6: 2.0 mg l ⁻¹ TOP	50.1b	70.0b, B	33.5b, A

*Different small letters in the columns indicate differences ($P < 0.05$) between the pre-treatments; the different block capitals in the rows indicate differences between the regeneration treatments. R1: regeneration medium with 0.2 mg l⁻¹ TDZ; R5: regeneration medium with 5.0 mg l⁻¹ BA.

Table 3
Effects of pre-treatments and regeneration medium on the number of regenerated shoots*

Pre-treatments	Main effect of pre-treatment: number of regenerated shoots	Effect of regeneration medium: number of regenerated shoots	
		R1	R5
No. 1: cytokinin-free	6.1a	6.4a, A	5.9ab, A
No. 2: 0.5 mg l ⁻¹ BA	8.3ab	11.9bc, B	4.6a, A
No. 3: 0.5 mg l ⁻¹ TOP	12.4c	15.1c, B	8.9c, A
No. 4: 1.0 mg l ⁻¹ TOP	10.3bc	14.1c, B	6.4bc, A
No. 5: 1.5 mg l ⁻¹ TOP	10.0bc	12.5bc, B	7.5bc, A
No. 6: 2.0 mg l ⁻¹ TOP	9.9bc	13.3c, B	6.5bc, A

* Different small letters in the columns indicate differences ($P < 0.05$) between the pre-treatments; different block capitals in the rows indicate differences between the regeneration treatments. R1: regeneration medium with 0.2 mg l⁻¹ TDZ; R5: regeneration medium with 5.0 mg l⁻¹ BA.

A range of TOP concentrations from 0.5 to 1.5 mg l⁻¹ in the pre-treating medium caused a significant decrease in the rate of vitrified shoots compared to the post-effect of BA-containing or cytokinin-free pre-treating media considering the main effects of pre-treatments (Table 2). When the concentration of TOP was raised to 2.0 mg l⁻¹, the rate of vitrification increased significantly but did not reach the level observed after pre-treatment on BA-containing or cytokinin-free pre-treating media. This favourable effect of TOP was very pronounced when TDZ was used in the regeneration medium (R1).

When the regeneration was induced on R5 regeneration medium, which contained 5.0 mg l⁻¹ BA, the vitrification was much lower than on R1 medium. It was the highest after pre-treatments on cytokinin-free medium. The application of TOP or BA in the pre-treating media resulted in a decrease in vitrification, which was significantly the lowest after pre-treating on medium with 0.5 mg l⁻¹ TOP.

When regeneration was induced on R1 regeneration medium, the most shoots developed after pre-treatment on media with TOP, while pre-treatment with BA slightly decreased the shoot number per explant. Cytokinin-free pre-treatment caused a significant decrease in the number of shoots regenerated. If regeneration was induced on R5 regeneration medium, the tendency was similar and the best results were achieved after pre-treatment on medium containing 0.5 mg l⁻¹ TOP (Table 3).

Considering the main effects of the pre-treatments, the organogenetic index (OI) was the highest after pre-treatment with TOP in a concentration range between 0.5 and 1.5 mg l⁻¹ (Fig. 2), when histological studies on pre-treated leaves proved that the leaf tissues had a juvenile-like, less differentiated character (Fig. 1C–E). When regeneration was induced on R5 regeneration medium, the OI was considerably higher after pre-treatment with 0.5 mg l⁻¹ TOP and was the lowest after pre-treatment on media with BA or without cytokinin. The OI was the highest after pre-treatment with 1.0 or 1.5 mg l⁻¹ TOP when regeneration was induced on R1 regeneration medium, due to the lowest vitrification percentage in these treatments. After pre-treatment with BA the OI was zero because of the 100% vitrification rate.

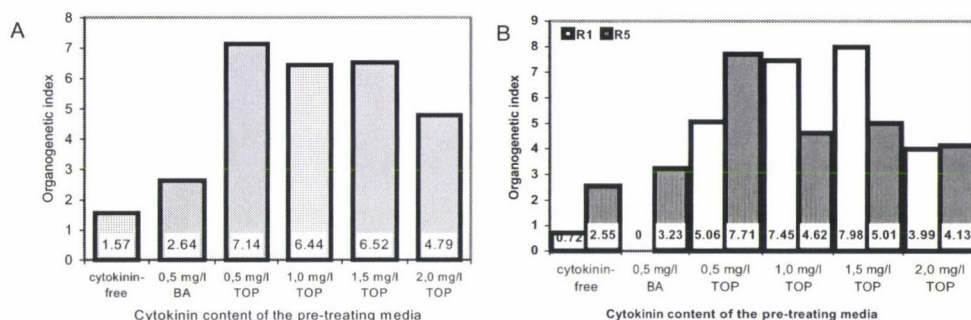


Fig. 2. Effect of pre-treatments (A) and regeneration media (B) on the organogenetic index. [Organogenetic index = (regeneration percentage - vitrification percentage) × shoot number per organogenetic explant/100] (R1: regeneration medium with 0.2 mg l⁻¹ TDZ; R5: regeneration medium with 5.0 mg l⁻¹ BA)

Discussion

In this paper the favourable effect of TOP on the morphogenic activity of *in vitro* apple leaves was demonstrated. TOP was reported to have a similar effect on micropropagation and shoot regeneration in other plant species (Kubaláková and Strnad, 1992; Werbrouck et al., 1996; Strnad et al., 1997) but no publications have been found on tree species.

It can be concluded from the histological results that TOP applied in a concentration range from 0.5 to 1.5 mg l⁻¹ caused the leaves to have a less differentiated, juvenile-like structure. Sriskandarajah and Goodwin (1998) reported a similar histological structure in the leaf tissues after conditioning apple shoots for several days in liquid medium. However, after the other pre-treatments in the present experiments, the leaf tissue was differentiated to varying extents. The differentiation was the least pronounced after treatment with 2.0 mg l⁻¹ TOP and was the most obvious after cytokinin-free treatment.

TDZ has often been used to stimulate shoot regeneration from leaves of apple (van Nieuwkerk et al., 1986; Fasolo et al., 1988; 1989; Theiler-Hedtrich and Theiler-Hedtrich, 1990; Sriskandarajah et al., 1990; Swartz et al., 1990; Korban et al., 1992), but it has several undesirable side-effects such as fasciated and vitrified shoots (van Nieuwkerk et al., 1986; Huettelman and Preece, 1993). In the present experiments TDZ increased the number of regenerated shoots compared to BA (Table 3) but the rate of vitrified shoots was also significantly higher than on regeneration medium containing BA (R5) as cytokinin source (Table 2).

Theiler-Hedtrich and Theiler-Hedtrich (1990) studied the effect of TDZ and BA on shoot regeneration from apple leaves of several rootstocks and scions and reported that the effect of 0.2 mg l⁻¹ TDZ was comparable to that of 5.0 mg l⁻¹ BA considering the number of regenerated shoots. In the present experiments this was confirmed only when regeneration was induced after cytokinin-free pre-treatment. After other pre-treatments TDZ induced significantly more shoots than BA. In general, the number of regenerated shoots per explant increased significantly after pre-treatments with TOP (Table 3).

The application of TOP in a range of concentration between 0.5 and 1.5 mg l⁻¹ for the pre-treatment of apple shoots caused a juvenile-like structure in the leaves and increased the organogenetic index. However, the post-effects of the different concentrations of TOP depended on the type of cytokinins used in the regeneration media.

Considering the histological structure of the upper two leaves used for regeneration and their regeneration capacity: the number of regenerated shoots and the rate of vitrification showed a good correlation. These results confirmed that growth regulators applied before the regeneration phase could modify the organogenetic potential of leaf tissues, as reported by others (McHughen et al., 1989; Swartz et al., 1990; Ferradini et al., 1996; Sriskandarajah and Goodwin, 1998).

Considering the organogenetic index, the results proved the effect of cytokinins in the stage prior to regeneration and their interaction with the cytokinins applied in the organogenesis phase in accordance with the results achieved in other plant species (Antonelli and Druart, 1990; Swartz et al., 1990; Famiani et al., 1994).

These results suggest that meta-topolin, as a new source of cytokinin, could be used to improve the regeneration efficiency from apple leaves, because it resulted in a juvenile-like, less differentiated leaf tissue structure and increased the morphogenic activity of apple leaves. The concentration proposed for the conditioning of apple will depend on the cytokinin content of the regeneration medium because of the interaction between the cytokinin content of the pre-treating medium and that of the regeneration medium. This type of interaction was proved to modify the organogenetic index. Experiments with meta-topolin to study its cytokinin effect in the regeneration medium in comparison with BA or TDZ are underway in our laboratory.

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EFFECTS OF SALINITY ON WATER USE EFFICIENCY AND ITS COMPONENTS IN CHICKPEA (*CICER ARIETINUM* L.)

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In order to investigate the effects of salinity on water use efficiency (WUE) and its components, i.e. transpiration efficiency (TE), uptake efficiency (UE) and harvest index (HI), in chickpea, four chickpea cultivars were grown in pots containing soils with 0.8 (C=control), 2 (S₁) and 3.9 (S₂) dSm⁻¹ salinity. At S₁, the WUE of all cultivars increased, but subsequently decreased with increasing salinity. The relative contribution of TE to the sum of squares of WUE was relatively higher than UE and HI. Therefore, the TE component had a more crucial improving effect on WUE than the other two WUE components. At the S₂ level, UE and HI were lower than in the control. Path analysis revealed that the simultaneous selection of non-stressed cultivars for higher TE and HI, and of salt-stressed cultivars for better TE and UE should be practised to improve WUE under non-stressed and high stress conditions, respectively. With respect to ion contents, the screening of salt-subjected cultivars for higher K⁺ content in the roots might result in an increase in both the total dry matter (TDM) and WUE of chickpea under stress conditions. Additionally, the selection of non-stressed cultivars on the basis of higher shoot Ca²⁺ concentration might stimulate the TDM and WUE of chickpea under non-stressed conditions.

Key words: chickpea, salinity, water use efficiency, transpiration efficiency, uptake efficiency, harvest index

Introduction

As in other glycophytes, the growth and grain yield (GY) of chickpea is reduced by salinity (Soussi et al., 1998; 1999). It is generally believed that the transpiration rate and consequently the total water used (TWU) show decreasing trends with increasing salt intensity (Abbas et al., 1991; Rajasekaran et al., 1997; Redmann et al., 1994; Richardson and McCree, 1985; Robinson et al., 1997). If GY and TWU decrease at a similar rate, the water use efficiency (WUE), i.e. the GY/TWU ratio, is not affected by salinity. So far, little information is available about the responses of chickpea to salinity in respect of WUE and its components. Furthermore, no reports have been published about the relative contribution of each WUE component to an increase in WUE in salt-stressed and non-stressed chickpea. Therefore, a greenhouse pot experiment was conducted, using four chickpea cultivars.

Materials and methods

Four local chickpea (*Cicer arietinum* L.) cultivars (Ahar, Ardabil, Shabestar and Maragheh) were obtained from the Agricultural Research Centre, Maragheh, Iran. The soil was salinized with three NaCl levels to obtain EC values of 0.8 (C=control), 2 (S₁) and 3.9 dSm⁻¹ (S₂), using the method of Richter et al. (1995).

In a factorial experiment, based on a randomized complete block design with three replications, 4 seeds from each cultivar were sown in each weighed pot. After seedling emergence, 3 seedlings were removed from each pot, leaving only one. The pots were weighed every 3 days and amounts of water equal to the loss in weight were added. The serial destructive plant samples were taken from each pot at intervals of 6 days, starting at 27 days after sowing, up to 10 days before grain harvesting. At each sampling, the fresh and oven-dried weight of root and shoot were separately determined. The water loss at the sampling intervals was estimated, using regression analysis, 27 days after sowing. At the final harvest, GY was determined.

Total transpired water (TWT) and total evaporated water were estimated, using the data for planted and unplanted pots (3 pots for the control and 6 pots for salinized soils at each replication). WUE was calculated as the GY/TWU ratio. TWU includes both TWT and total evaporated water. The three components of WUE are uptake efficiency (UE), transpiration efficiency (TE) and harvest index (HI), which are defined as follows (Ehdaie and Waines, 1993):

$$UE = TWT/TWU$$

$$TE = TDM/TWT$$

$$HI = GY/TDM$$

where TDM = root dry matter (RDM) + shoot dry matter (SDM) + GY. Thus WUE can be expressed as:

$$WUE = GY/TWU = (TWT/TWU) \times (TDM/TWT) \times (GY/TDM)$$

Taking logarithms of the expression yields the following equation:

$$Y = X_1 + X_2 + X_3$$

in which $Y = \log (WUE)$, $X_1 = \log (UE)$, $X_2 = \log (TE)$ and $X_3 = \log (HI)$. The contribution of each X_i term to the sum of squares of Y was estimated according to the method of Moll et al. (1982).

At final harvest, the concentrations of Na⁺, Cl⁻, K⁺ and Ca²⁺ in both root and shoot were separately determined, as detailed by Mamo et al. (1996).

The data were analysed using various univariate and multivariate statistical methods with SAS software (SAS, 1986).

Results

The results of multivariate analysis of variance revealed that the effects of salt on the measured attributes were highly significant (Table 1). The responses of the cultivars to salinity were significantly different. The cultivar \times salt interaction was also significant, proving that the differences in cultivar responses varied with increasing salt concentration in the medium. These results were confirmed by univariate analysis of variance (data not presented).

As shown in Table 2, the growth and consequently the TDM of cultivar V₁ were stimulated by the first level of salt stress (S₁). This was not true for the other three cultivars. At the higher stress intensity (S₂), the TDM of all cultivars was lower when compared with the control (C). Cultivar V₂ appears to be the most tolerant cultivar, according to TDM.

Table 1
Multivariate analysis of variance for measured attributes

S.V.	Numerator df	Denominator df	Wilk's lambda value	F
Replication	18	28	0.142	2.56**
Salt	18	28	222×10^{-7}	327**
Cultivar	27	41.53	96×10^{-6}	34**
Cultivar×salt	54	75.98	55×10^{-8}	22**

** : Significant at $p < 0.01$

In the control, the TWT value for V_3 was lower than that of the other cultivars (Table 2). The value of TWT decreased with increasing NaCl doses. At all salt levels, the value of TWT was statistically different for the tested cultivars. As expected, the value of TWU for stressed cultivars was lower than for the control (Table 2). Under high stress conditions, the most tolerant cultivar possessed higher TWU than the others.

The S_1 level of salinity induced an increase in WUE in all the cultivars (Table 2), while the S_2 level resulted in a decrease in WUE. In the presence of a high NaCl dose, salt-tolerant and salt-sensitive (V_4) cultivars had statistically similar WUE, which was of a higher order than that of the other cultivars. At the C and S_1 levels, the value of WUE for V_3 was higher than for the other cultivars.

Table 2

Means of root (RDM -g/plant), shoot (SDM -g/plant) and total dry matter (TDM -g/plant), grain yield (GY -g/plant), total transpired water (TWT -cm³/plant), total water used (TWU -cm³/plant), water use efficiency (WUE -g/l), uptake efficiency (UE), transpiration efficiency (TE -g/l) and harvest index (HI)

Treatment combination	RDM	SDM	GY	TDM	TWT	TWU	WUE	UE	TE	HI
CV ₁	11.81	20.10	9.33	41.24	11280	17720	0.526	0.636	3.65	0.226
CV ₂	11.82	22.45	9.32	43.59	10850	17340	0.537	0.625	4.02	0.213
CV ₃	12.44	24.22	10.32	46.98	9490	16040	0.643	0.591	4.95	0.219
CV ₄	12.64	22.39	9.78	43.81	10280	16420	0.595	0.626	4.26	0.223
S_1V_1	12.61	21.12	8.95	42.69	8652	14780	0.606	0.585	4.93	0.201
S_1V_2	12.04	19.93	9.03	41.00	8770	14660	0.616	0.598	4.67	0.220
S_1V_3	11.51	20.30	8.77	40.58	7860	13150	0.666	0.597	5.16	0.216
S_1V_4	11.80	20.31	9.01	41.13	7776	13890	0.649	0.560	5.29	0.219
S_2V_1	9.14	14.22	5.99	29.37	7647	14430	0.415	0.530	3.84	0.204
S_2V_2	9.74	16.54	6.53	32.82	7534	15400	0.424	0.489	4.35	0.199
S_2V_3	8.41	12.51	5.49	26.42	7445	14060	0.390	0.529	3.54	0.208
S_2V_4	8.36	12.34	5.39	26.10	6776	12790	0.421	0.530	3.85	0.206
LSD ($p < 0.01$)	0.07	0.10	0.05	0.22	49.15	82.7	0.004	0.017	0.163	0.002

C = control; $S_1 = 2 \text{ dSm}^{-1}$; $S_2 = 3.9 \text{ dSm}^{-1}$; $V_1 = \text{Ahar}$; $V_2 = \text{Ardabil}$; $V_3 = \text{Shabestar}$; $V_4 = \text{Maragheh}$

The highest UE was recorded in non-stressed V_1 , V_2 and V_4 cultivars (Table 2). A low dose of NaCl had no significant effect on the UE value of V_3 . At a high dose of NaCl, V_2 had the lowest UE. The path analysis results revealed that under non-stressed conditions, the direct effect of UE on WUE was statistically negligible, but this attribute had a considerable indirect effect through TE (Table 3). At S_2 , the direct positive effect of UE on WUE was counterbalanced by the indirect effect via TE. At this salt level, the relative contribution of UE to the sum of squares of WUE ($\Sigma X_i Y / \Sigma Y^2$) was lower than that of TE, but higher than that of HI (Table 3). This was not true under non-stressed conditions.

The TE and WUE were similarly affected by salt stress, since the $\Sigma X_i Y / \Sigma Y^2$ ratio for TE was the highest (Table 3). The TE directly favoured WUE, especially at the S_2 level (Table 3). The indirect effects of TE via UE and through HI were positive under C and S_2 conditions, respectively.

In contrast with the control, the HI value for cultivars subjected to high salt was lower (Table 2). At the C and S_2 levels, the HI was lower in V_2 than in the other three cultivars. At the S_1 level, the HI value was similar for V_2 and V_4 . In the absence of salt stress, WUE was directly affected by HI, but in the presence of high salt stress it was not affected (Table 3).

The results of elemental analysis indicated that the concentrations of Na^+ and Cl^- in the root and shoot (RNa, RCl, SNa and SCl, respectively) were increased due to increasing salt levels (Table 4). This increase was correlated with reduced K^+ and Ca^{2+} contents in the root and shoot (RK, RCa, SK and SCa, respectively) (Table 4). Under high salt-stress conditions, TDM was negatively linked to both Na^+ and Cl^- contents, but positively to both K^+ and Ca^{2+} concentrations (data not shown). The TDM, WUE and TE attributes of high salt-treated plants were positively affected by RK (Table 5). In the case of non-salt-treated plants, this was true for SCa. As seen in the table, some of the other ions had a favourable effect on some but not all of the TDM, TE and WUE attributes.

Table 3

Path analysis of effects of UE (uptake efficiency), TE (transpiration efficiency) and HI (harvest index) and their relative contributions to the sum of squares of water use efficiency ($\Sigma X_i Y / \Sigma Y^2$), using the model $Y = X_1 + X_2 + X_3$

Salt level	Trait	Direct effect or S_x / S_y	Indirect effect via			Correlation (Total effect)	$\Sigma X_i Y / \Sigma Y^2$
			UE	TE	HI		
C ⁺	UE	0.07	1	-0.96	0.04	-0.85**	0.064
	TE	0.89**	0.07	1	-0.06	0.90**	0.732
	HI	0.22**	-0.02	-0.25	1	-0.05	0.204
	UE	0.67**	1	-1.09	0.05	-0.37	0.211
S_2	TE	1.21**	-0.60	1	0.09	0.70**	0.760
	HI	-0.09	0.61	-1.16	1	-0.64*	0.029

$R^2=0.96$; *: Significant at $p<0.05$; **: Significant at $p<0.01$; ⁺: C = control; $S_2 = 3.9 \text{ dSm}^{-1}$; Ridge regression was used because of collinearity between the independent variables (Belsley et al., 1980)

Table 4
Mean ion contents (Na^+ , Cl^- , K^+ and Ca^{2+}) in root (R) and shoot (S) (mg g^{-1} dry weight)

Treatment combination	RNa	RCl	RK	RCa	SNa	SCl	SK	SCa
CV ₁	2.77	2.59	53.53	27.36	3.70	2.87	31.39	16.10
CV ₂	2.31	2.09	47.46	24.64	3.57	3.45	33.41	16.42
CV ₃	2.55	2.20	45.33	23.78	3.81	3.28	32.33	16.99
CV ₄	2.53	2.13	44.10	23.42	3.71	3.13	31.60	16.73
S ₁ V ₁	5.46	40.38	50.10	22.48	8.29	57.07	29.81	15.09
S ₁ V ₂	5.20	39.53	43.33	21.47	9.68	58.59	30.20	15.18
S ₁ V ₃	6.56	47.16	41.22	21.00	8.71	62.60	29.84	15.13
S ₁ V ₄	6.63	50.20	41.93	21.90	8.59	65.07	29.92	15.06
S ₂ V ₁	10.93	54.20	27.06	14.39	19.20	88.47	25.55	12.21
S ₂ V ₂	11.19	56.90	29.14	15.13	17.59	82.97	26.17	13.00
S ₂ V ₃	12.44	60.89	22.51	12.17	20.49	93.06	24.46	12.10
S ₂ V ₄	12.00	59.05	22.15	11.59	20.92	95.49	22.51	11.40
LSD ($p < 0.01$)	0.21	1.00	0.88	0.46	0.34	1.58	0.52	0.35

C = control; S₁ = 2 dSm⁻¹; S₂ = 3.9 dSm⁻¹; V₁ = Ahar; V₂ = Ardabil; V₃ = Shabestar; V₄ = Maragheh

Discussion

Although the growth, and consequently the TDM, of all tested cultivars were diminished by a high salt level, this reduction was most apparent in V₄ (Table 2). The V₂ and V₁ cultivars showed the best and 2nd best salt tolerance, respectively. Genetic variation for salt tolerance was also reported in chickpea (Ashraf and Waheed, 1992; Gholipour et al., 2000; Mamo et al., 1996; Richter et al., 1999). Higher salt tolerance was found to be correlated with higher K⁺ and Ca²⁺ contents, but with lower Na⁺ and Cl⁻ concentrations in both the root and shoot (data not presented). These findings are in agreement with previous observations in seedlings of other cultivars (Gholipour et al., 2000).

There was an inverse relationship between salt level and TWT ($r = -0.96^{**}$). Some of the salt-resulted decrease in TWT was due to salinity-induced early maturity. Similarly to drought stress, the reduction in TWT with increasing salt stress is associated with reduced stomatal conductance for H₂O exchange (Robinson et al., 1997). At the S₂ level, TDM was significantly correlated with TWT and TWU ($r=0.60^*$ and $r=0.89^{**}$, respectively). A strong correlation between TDM and TWU was previously reported for many crops (Ehdaie and Waines, 1993; Hubick et al., 1986; Ismail and Hall, 1992).

The increase in WUE recorded due to the first level of salt stress (Table 2) could be attributed to the slight closure of the stomata (Letey, 1993). Similar findings have been reported for *Atriplex* by Glenn and Brown (1998). On the other hand, a consistent decrease in WUE due to increasing salt level was observed in alfalfa (Khan et al., 1998), safflower (Alyari et al., 2000) and lettuce (Ruiz-Lazano et al., 1996).

The present results revealed that UE was inversely related to salt intensity ($r = -0.96^{**}$), proving that a salt-resulted decrease in TWT is followed by an

increase in evaporated water. Considering the inverse relationship between UE and RDM under high saline conditions ($r = -0.82^{**}$) and the negligible relationship under non-saline conditions ($r = 0.03$), it is clear that in the presence and absence of salt stress, better UE is not commensurate with higher RDM and that, as reported by Passioura (1972), other characters, including internal organizations and the size of the vascular system, are more important than RDM. Accordingly, it seems that screening cultivars for higher RDM will not result in enhanced UE and thus better WUE.

Although the direct effect of UE on WUE was significant at S_2 (Table 3), the selection of salt-subjected cultivars for higher UE may not result in enhanced WUE, because this direct positive effect was counterbalanced by a decrease in TE.

In arid and semi-arid regions in which salt limits crop growth (Richter et al., 1995), crops experience some water stress, especially during the irrigation intervals. Any effect that salinity might have, should be most obvious during the watering intervals (McCree and Richardson, 1987). Therefore, under simultaneous salt and drought stresses, UE, i.e. the overall ability of the plant to absorb water from the soil and to reduce soil evaporation, traits that are associated with root characteristics and of early growth habit and canopy closure, is more important than during salt stress alone. Accordingly, field experiments with a large number of cultivars/lines are needed for screening for higher UE. Note that, under field conditions, mathematical equations should be used to estimate TWT and TWU.

The TE reflects the ratio of assimilation rate to water used. The $\Sigma X_i Y / \Sigma Y^2$ ratio for TE was the highest under both saline and non-saline conditions (Table 3). Furthermore, the direct and total positive effects of TE were higher compared with those of other WUE components. For this reason, screening for higher TE might have a greater positive effect on WUE than the other two components under both salt and non-salt stress conditions. However, as shown in Table 3, selection for TE alone might result in a reduction in the other WUE components. Therefore, the simultaneous screening of salt-stressed cultivars for better TE and UE should be practised to improve WUE under stress conditions. In addition, the simultaneous selection of non-stressed cultivars for higher TE and HI may result in enhanced WUE under non-stressed conditions.

There was a significant correlation between WUE and TDM under non-stressed conditions ($r = 0.87^{**}$), but not under high stress conditions ($r = 0.50$), indicating that higher WUE is not always commensurate with higher TDM. Therefore, an improvement of both WUE and TDM attributes must be considered. Accordingly, in this experiment, the effects of various ions on WUE, TDM and TE were calculated (Table 5). These results showed that the screening of non-salt-subjected cultivars for higher SCa should result in enhanced productivity (TDM and WUE) in chickpea under non-salt-stressed conditions. Moreover, the selection of salt-stressed cultivars on the basis of higher RK may improve productivity under stress conditions.

Table 5

Direct and total effects of tested ions (Na^+ , Cl^- , K^+ and Ca^{2+}) in root (R) and shoot (S) on transpiration efficiency (TE), water use efficiency (WUE) and total dry matter (TDM)

Salt level	Trait	Direct effect on TE	Total effect on TE	Direct effect on WUE	Total effect on WUE	Direct effect on TDM	Total effect on TDM
C^+	RNa	0.06	-0.25	0.09	-0.08	0.03	-0.38
	RCl	0.05	-0.52	0.02	-0.43	0.04	-0.61*
	RK	-0.19**	-0.74**	-0.26**	-0.74**	-0.17**	-0.75**
	Rca	-0.28**	-0.74**	-0.33**	-0.73**	0.27**	-0.76**
	SNa	0.51**	0.62*	0.48**	0.72**	0.48**	0.50
	SCl	0.12**	0.47	0.01	0.26	0.17**	0.61*
	SK	0.24**	0.19	0.07	-0.06	0.31**	0.34
	SCa	0.13**	0.89**	0.12**	0.87**	0.15**	0.87**
S_2	RNa	-0.27**	-0.64*	-0.55**	-0.65*	-0.07	-0.75**
	RCl	0.21**	-0.45	0.02	-0.56*	0.07	-0.59*
	RK	0.24**	0.80**	0.29**	0.56*	0.18**	0.97**
	RCa	-0.25**	0.72**	-0.37**	0.43	0.06	0.95**
	SNa	-0.39**	-0.83**	-0.18**	-0.43	-0.25**	-0.98**
	SCl	-0.07	-0.78**	0.26	-0.39	-0.18**	-0.97**
	SK	0.10*	0.75**	0.12**	0.54	0.09	0.92**
	SCa	0.16**	0.76**	0.05	0.35	0.20**	0.95**

^+C = control; S_2 = 3.9 dSm $^{-1}$; *: Significant at $p < 0.05$; **: Significant at $p < 0.01$

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EFFECTS OF THE LENGTH OF INCUBATION PERIOD AND HERBICIDES ON NITROGENASE ACTIVITY OF PEA (*PISUM SATIVUM* L.)

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In a pot experiment the effects of the length of incubation period and the pre-emergence application of terbutryn/terbuthylazine at 2.80 kg a.i. ha⁻¹ or post-emergence application of bentazone at 2.88 kg a.i. ha⁻¹ on the nitrogenase activity in intact pea plants were measured *in situ* by the acetylene reduction assay. An incubation period of 10 min resulted in the highest nitrogenase activity. As the length of the incubation period increased to 30 or 60 min the total as well as the specific nitrogenase activity decreased. Terbutryn/terbuthylazine decreased the total nitrogenase activity at all three (i.e. vegetative, flowering and seed-filling) stages, whereas bentazone resulted in a significant decrease at the flowering stage only. However, terbutryn/terbuthylazine-treated plants had the highest specific nitrogenase activity both at the flowering and seed-filling stages.

Key words: herbicides, pot experiment, incubation period, nitrogenase activity, pea, *Pisum sativum* L.

Introduction

The acetylene reduction assay is a simple, rapid, cheap and sensitive method of measuring nitrogenase activity (Hardy et al., 1968; 1973; Masterson and Murphy, 1980; Turner and Gibson, 1980; Vessey, 1994). However, there are some demerits of this method, such as plant disturbance (Minchin et al., 1986), washing of roots (Mague and Burris, 1972) and nodule detachment (Hardy et al., 1973), which are reported to decrease nitrogenase activity. Moreover, it is not possible to recover all the nodules, especially under field conditions (Witty and Minchin, 1988). To avoid these problems it is possible to grow the plants in pots in a porous medium, such as perlite or vermiculite, and to use the pots themselves as the incubation vessels by making them gas-tight and thus to measure the nitrogenase activity *in situ*. In the present studies nitrogenase activity was measured on intact plants *in situ* to study the effect of three incubation periods (10, 30 and 60 min) in pea (*Pisum sativum* L.).

Biological nitrogen fixation may play an important role in farming systems. However, many factors may limit the efficiency of biological nitrogen fixation in legumes. One of these factors in modern agriculture may be the use of herbicides. Linuron in soybean (Rennie and Dubetz, 1984), bentazone in kidney bean (Bethlenfalvay et al., 1979; Schnelle and Hensley, 1990), alachlor,

metribuzin and trifluralin in soybean (Mallik and Tesfai, 1985), chlorosulfuron in alfalfa (Mårtensson and Nilsson, 1989), oxyfluorfen, linuron, metribuzin and oxadiazon in lentil (Sandhu et al., 1991) and metribuzin in lentil (Sprout et al., 1992) have been shown to have adverse effects on nitrogen fixation. In the present paper the effects of two herbicides, i.e. terbutryn/terbuthylazine and bentazone, on nitrogenase activity were studied.

Materials and methods

Location

The studies were carried out at Henfaes Farm, University of Wales, Bangor, Gwynedd, United Kingdom in a walk-in growth room with a day/night temperature of 18°/9°C and a 16 h photoperiod. The photosynthetic photon flux density was $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the plants during the growing season and relative humidity was maintained at 70%.

Treatments and experimental design

The three treatments tested were: a) pre-emergence herbicide terbutryn/terbuthylazine (as 'Opogard' having 350 g terbutryn + 150 g terbuthylazine/litre) at 2.80 kg a.i./ha; b) post-emergence herbicide bentazone (as 'Basagran' having 480 g bentazone/litre) at 2.88 kg a.i./ha; and c) unsprayed control. These rates are double the recommended field application rates for both the herbicides. Terbutryn/terbuthylazine was applied immediately after sowing and bentazone was sprayed 25 days after sowing (DAS). Before spraying bentazone a pea leaf wax test (Gane et al., 1984; PGRO, 1993) was performed using a 1% solution of Crystal Violet to decide whether the plants had sufficient leaf wax to allow the application of this herbicide without damaging the peas. Terbutryn and terbuthylazine belong to the triazine group whereas bentazone is a benzothiadiazole (Schmidt, 1997). The measurements of nitrogenase activity were made at three growth stages, i.e. the vegetative stage (30 DAS), the flowering stage (64 DAS) and the seed-filling stage (80 DAS). The growth key codes for the respective stages are 103, 204 and 207, as described by Knott (1987). The number of replicates measured at these three stages were 8, 5 and 5, respectively. When measuring nitrogenase activity gas samples were taken after 10, 30 and 60 min incubation periods. The data were analysed in a factorial randomised complete block design, considering herbicide treatments and incubation period as two factors, by the analysis of variance (ANOVA) method, using the Minitab statistical package version 10.51.

Plant growth conditions

The experiment was sown on 18 November 1996. The seeds were not treated with fungicide. Prior to sowing the seeds were soaked in a liquid *Rhizobium* culture for 1 h. Two seeds of pea (*Pisum sativum* L.) cv. Rex, a normal-leaved variety, were sown in perlite in 800 cm³ plastic pots. These pots were about 12 cm high and 9.2 cm in diameter and had gas-tight screw fitting lids to enable the measurement of nitrogenase activity *in situ*. Thinning was done 11 days after sowing to retain only one plant per pot. These were inoculated with 4 cm³ 7-day old liquid culture of *Rhizobium leguminosarum* strain RCR 1045.

Until germination the pots were watered with tap water. After emergence the plants were watered with Long Ashton N-free nutrient solution (Hewitt, 1966) three days a week (Mondays, Wednesdays and Fridays) and with tap water on the other days. The plants were watered daily, except on the day when nitrogenase activity was measured, with as much water as they needed allowing no/very little drainage. The tap water was tested for nitrate content and was found to be nitrogen-free. The plants were given support by tying them to sticks attached to the outer side of each pot. Each pot had one thin stick to support the plant.

In situ measurement of nitrogenase activity

Nitrogenase activity was measured on intact plants using the acetylene reduction assay (Hardy et al., 1973; Masterson and Murphy, 1980; Turner and Gibson, 1980; Jonsson, 1988) under the same conditions in which the plants were grown. Thus, the incubation temperature was 18°C for all stages of measurement. Measurements were made 3–4 h after the start of the photoperiod. The pots were made gas-tight by replacing their lids and plugging all holes, including the drainage holes, with plasticine. The partially cut lids had a hole in the centre for the plants to pass through. This hole was also sealed with plasticine. Approximately 40 cm³ of acetylene was injected into the pots, so that the total available pore space, as determined previously by the displacement method, had a concentration of 10% (v/v) acetylene. The acetylene was injected with a 50 cm³ syringe, having an 8.5 cm long needle, through a small hole in the lid, and as the pressure built up the same amount of air was allowed to escape through another needle inserted in the lid for this purpose. After injecting the acetylene both the needles were removed and these small holes were immediately plugged with plasticine.

The incubation periods were 10, 30 and 60 min, after which gas samples of 0.5 cm³ were taken in 1 cm³ syringes (Sherwood Medical, Northern Ireland) fitted with 23G × 1" needles. These samples were run in a portable gas chromatograph (Swedish University of Agricultural Sciences, Stencil, Umeå) calibrated with known ethylene concentrations; the internal temperature was adjusted to 30°C. The gas chromatograph was fitted with a Durapak column. Dry air (50 ml min⁻¹) was used as the carrier gas. The three peaks (resistant, ethylene and acetylene) were recorded on graph paper with a flat mini recorder (Sekonic SS-100F model; Sekonic Co. Ltd, Tokyo, Japan). The samples to be analysed were stored in syringes in the laboratory by putting the needles into rubber stoppers to avoid any gas leakage. The silicon septum of the gas chromatograph was changed after 15–20 injections. As the pots contained perlite the volume available for the diffusion of gases (acetylene and ethylene) in the pots was determined by a displacement method and this volume was used for calculating nitrogenase activity. Nitrogenase activity was expressed as μmol C₂H₄ produced plant⁻¹ h⁻¹ (known as total nitrogenase activity) or μmol C₂H₄ produced g⁻¹ nodule fresh wt h⁻¹ or μmol C₂H₄ produced g⁻¹ nodule dry wt h⁻¹. The nitrogenase activity expressed per unit nodule weight (fresh or dry) is known as specific nitrogenase activity.

Results

Effects of the length of incubation period

At all stages of measurement an incubation period of 10 min gave the highest nitrogenase activity and an increase in the length of the incubation period resulted in a marked decrease in total nitrogenase activity (Table 1) and specific nitrogenase activity (Tables 2 and 3). Statistically, an incubation period of 60 min gave significantly lower nitrogenase activity values compared to an incubation period of 10 min.

Effects of herbicides on nitrogenase activity

In both the herbicide treatments the total nitrogenase activity increased over time (Table 1). However, in the untreated control plants it increased only up to the flowering stage and then decreased. Terbutryn/terbuthylazine resulted in a significant decrease in total nitrogenase activity at all stages. The decrease was 43, 80 and 28% at the vegetative, flowering and seed-filling stages,

respectively. Bentazone resulted in a significant decrease (41%) in total nitrogenase activity at the flowering stage only. Conversely, specific nitrogenase activity, when expressed on the basis of nodule fresh (Table 2) or dry weight (Table 3) was the highest in terbutryn/terbuthylazine-treated plants at both flowering and seed-filling and was significantly higher than in bentazone-treated or control plants.

Length of incubation period \times herbicide interaction

The herbicide \times length of incubation period interaction was significant at the flowering and seed-filling stages when activity was expressed on a nodule fresh or dry weight basis (Tables 2 and 3). The effects of terbutryn/terbuthylazine were significant after an incubation period of 10 min, but not after 30 or 60 min. Though the total nitrogenase activity at all three stages was higher in control and bentazone-sprayed plants after an incubation period of 10 min, the interaction was not significant (Table 1).

Table 1
Effect of the length of incubation period (min) and herbicides on total nitrogenase activity ($\mu\text{mol C}_2\text{H}_4 \text{ plant}^{-1} \text{ h}^{-1}$) at different growth stages in peas

Herbicide	Length of incubation period			
	10	30	60	Mean
<i>Vegetative stage</i>				
	Total nitrogenase activity			
Terbutryn/terbuthylazine 2.80 kg ha ⁻¹	6.1	1.5	0.5	2.7
Bentazone 2.88 kg ha ⁻¹	9.3	4.5	2.6	5.4
Unsprayed control	8.5	3.6	2.1	4.7
Mean	8.0	3.2	1.7	—
<i>Flowering stage</i>				
Terbutryn/terbuthylazine 2.80 kg ha ⁻¹	11.5	3.6	2.3	5.8
Bentazone 2.88 kg ha ⁻¹	24.1	16.1	10.5	16.7
Unsprayed control	34.6	31.4	19.4	28.5
Mean	23.4	17.1	10.7	—
<i>Seed-filling stage</i>				
Terbutryn/terbuthylazine 2.80 kg ha ⁻¹	19.8	13.0	11.1	14.6
Bentazone 2.88 kg ha ⁻¹	32.4	23.4	14.1	23.6
Unsprayed control	30.7	19.1	11.5	20.4
Mean	27.6	18.5	12.2	—
LSD _{5%}	Vegetative stage Flowering stage Seed-filling stage			
Herbicides (H)	1.0	7.1	NS	
Incubation periods (I)	1.0	7.1	6.9	
H \times I interaction	NS	NS	NS	

NS = non-significant

Table 2

Effect of the length of incubation period (min.) and herbicides on specific nitrogenase activity ($\mu\text{mol C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule fresh wt h}^{-1}$) at different growth stages in peas

Herbicide	Length of incubation period			
	10	30	60	Mean
Specific nitrogenase activity				
<i>Flowering stage</i>				
Terbutryn/terbuthylazine 2.80 kg ha ⁻¹	1139	362	184	562
Bentazone 2.88 kg ha ⁻¹	85	50	31	55
Unsprayed control	107	97	59	87
Mean	443	170	91	—
<i>Seed-filling stage</i>				
Terbutryn/terbuthylazine 2.80 kg ha ⁻¹	81	37	25	47
Bentazone 2.88 kg ha ⁻¹	31	23	14	23
Unsprayed control	30	18	10	19
Mean	47	26	16	—
LSD _{5%}	Flowering stage		Seed-filling stage	
Herbicides (H)	185		4.7	
Incubation periods (I)	185		5.2	
H × I interaction	320		8.2	

Table 3

Effect of the length of incubation period (min) and herbicides on specific nitrogenase activity ($\mu\text{mol C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule dry wt h}^{-1}$) at different growth stages in peas

Herbicide	Length of incubation period			
	10	30	60	Mean
Specific nitrogenase activity				
<i>Flowering stage</i>				
Terbutryn/terbuthylazine 2.80 kg ha ⁻¹	2519	786	392	1232
Bentazone 2.88 kg ha ⁻¹	172	108	69	116
Unsprayed control	233	211	131	191
Mean	974	368	197	—
<i>Seed-filling stage</i>				
Terbutryn/terbuthylazine 2.80 kg ha ⁻¹	394	179	118	230
Bentazone 2.88 kg ha ⁻¹	157	116	71	114
Unsprayed control	153	94	53	100
Mean	235	129	81	—
LSD _{5%}	Flowering stage		Seed-filling stage	
Herbicides (H)	425		28	
Incubation periods (I)	425		31	
H × I interaction	736		49	

Discussion

Effects of incubation period on nitrogenase activity

The incubation period affected the rate of nitrogenase activity. In the present study three incubation periods (10, 30 and 60 min) were compared. The data (Tables 1–3) show that at all the measurement stages the nitrogenase activity decreased with an increase in the length of the incubation period. Hardy et al. (1968) reported that the rate of acetylene reduction by nodulated roots of soybean was constant up to 60 min and they also recommended that heavily nodulated roots should be assayed for a shorter time (30 min) since the rate for such samples decreases shortly after 60 min, presumably due to O₂ depletion. Using a flow-through gas system it has been shown that nitrogenase activity decreases shortly after the exposure of nodulated roots to acetylene (Minchin et al., 1986; Witty and Minchin, 1988). Measurements of nitrogenase activity and respiration at different external O₂ concentrations showed that the true cause of the acetylene effect was the O₂ limitation of bacteroid respiration, which was due to an increase in the resistance of the nodule to inward O₂ diffusion (Witty et al., 1984).

Effects of herbicides on nitrogenase activity

Terbutryn/terbuthylazine treatment resulted in low total nitrogenase activity (Table 1), though the specific nitrogenase activity was increased (Tables 2 and 3). As biological nitrogen fixation was the sole source of nitrogen for plant growth, the specific nitrogenase activity probably increased in an attempt to meet the nitrogen demand of the plant. Vadez et al. (1997) also reported lower total nitrogenase activity but higher specific nitrogenase activity at low phosphorus levels in kidney bean. In an earlier paper (Singh and Wright, 1999) it was reported that in terbutryn/terbuthylazine, bentazone and unsprayed control plants nodule dry weight was 9, 124 and 149 mg plant⁻¹ at flowering and 32, 201 and 192 mg plant⁻¹ at the seed-filling stage, respectively. Hence the decrease in total nitrogenase activity observed in terbutryn/terbuthylazine-treated plants was not due to a decrease in specific nitrogenase activity but to poor nodulation. Lower nodule dry weight per plant but no effect on specific nitrogenase activity was also reported after treatment with 0.60 kg ha⁻¹ terbutryn in lentils (Sandhu et al., 1991) and 1.6–3.2 kg ha⁻¹ prometryn in chickpea (Kumar et al., 1981). In soybean the number of nodules per plant in trifluralin-treated (2.8 kg ha⁻¹) plants was one-fifth of that in control plants, but the specific nitrogenase activity was 1.8 times greater in trifluralin-treated plants (Yueh and Hensley, 1993). This suggests that enhanced specific nitrogenase activity may be a means by which treated plants compensate for poor nodulation.

Bollich et al. (1985) and Ozair et al. (1990) also observed contradictory results when nitrogenase activity was expressed on a per plant and a per g nodule weight basis. As the nitrogenase activity per plant is a reflection of the overall effect of a herbicide on the symbiosis it is probably better to express nitrogenase activity on a per plant basis. Whole plant nitrogenase activity is the best basis, since expressing nitrogenase activity on the basis of nodule weight may lead to incorrect conclusions due to variation in the number of nodules per plant as well as in the volumes of active and senescent nitrogen-fixing regions (Herdina and Silsbury, 1990). Ozair et al. (1990) also recommended that nitrogenase activity should be considered on a per plant basis rather than a per nodule weight basis when evaluating the effects of herbicides on nitrogen fixation.

With an increase in age from flowering to seed-filling there was an improvement in the plant growth of terbutryn/terbuthylazine-treated plants (as reported in Singh and Wright, 1999). This might be due to the reduced effect of the herbicide, as with time the effect of herbicides may decrease or may be completely eliminated due to factors such as uptake and metabolism by plants, volatilisation, photodecomposition, leaching and degradation (Rao, 1987; Zimdahl, 1993; Anderson, 1996). This may explain why in this treatment the total nitrogenase activity increased between flowering and seed-filling (Table 1). Nitrogenase activity has been reported to decrease at seed-filling (Herridge and Pate, 1977; Minchin et al., 1980) as the result of competition between nodules and pods for photosynthate (see Neves and Hungria, 1987). At seed-filling the control plants had 2.72 g pod dry weight plant⁻¹, whereas at the same age terbutryn/terbuthylazine-treated plants were just at pod initiation and had only 0.27 g pod dry weight plant⁻¹. The lack of competition between nodules and pods for photosynthate may have resulted in higher nitrogenase activity at that stage in terbutryn/terbuthylazine-treated plants. At flowering the total nitrogenase activity of bentazone-treated plants was lower than that of the unsprayed control (Table 1). This might be due to the comparatively low weight of nodules per plant as compared to unsprayed control plants.

In conclusion, the length of the incubation period should be the shortest possible to avoid any acetylene-induced decline in nitrogenase activity. Only safe herbicides and low rates should be used to maintain higher rates of nitrogen fixation by legumes. When nitrogenase activity is expressed on a nodule fresh weight basis, the moisture content in the nodules may affect the calculations. So it is better to express it on a dry nodule weight basis. Best of all, however, nitrogenase activity should be expressed on a per plant basis.

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NITROGEN EFFECT ON THE INCIDENCE OF *STRIGA HERMONTHICA* (DEL.) BENTH IN UPLAND RICE

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Field trials were conducted in the dry (Experiment I) and wet (Experiment II) seasons of 1997 at Samaru (11°11' N, 7°38' E, 686 m above sea level) in the northern Guinea Savanna ecological zone of Nigeria to study the effects of nitrogen rates on the reaction of upland rice (*Oryza sativa* L.) varieties to *Striga hermonthica* (Del.) Benth. The results indicate that FARO 48, a variety normally susceptible to *Striga hermonthica*, exhibited resistance. FARO 11 exhibited tolerance, while FARO 38, FARO 46 and FARO 45 exhibited susceptibility. The application of 90 and 120 kg N/ha delayed and reduced *Striga* emergence on the crop, induced a low crop reaction score and produced grain yields that were the maximum or significantly higher than the least. No significant differences in *Striga* infestation were observed between nitrogen rates of 30–120 kg N/ha. The significant interaction between upland rice varieties and nitrogen rates indicates that the susceptible varieties require higher rates of nitrogen to ameliorate the effect of *Striga* compared with the resistant varieties.

Key words: incidence, upland rice, *Striga hermonthica*, northern Guinea savanna, resistance, tolerance, susceptibility

Introduction

Striga hermonthica has been reported to cause increasing devastation in upland rice production in the *Striga* endemic savanna ecological zones of Nigeria (Emechebe, 1991; Lagoke et al., 1993). This is due to the fact that more areas are being used for upland rice production, resulting in the consequent threat of a build up of *Striga* problems in the crop. There is therefore a need to combat the problem. Among various control methods, host plant resistance/tolerance as well as the application of nitrogen could play a major role in reducing *Striga* in upland rice. The term tolerance describes the ability of the host to withstand the effects of parasites that are already attached. On the other hand, the term resistance refers to the ability of a crop to prevent attachment of the parasite (above ground). Nitrogen rates between 120 kg N/ha (Adeosun 1990; Ngawa, 1991) and 280 kg N/ha (Robinson and Dowler, 1990) were found to reduce *Striga* damage on cereal crops such as maize and sorghum. The objective of this study was therefore to evaluate rates of nitrogen for the management of *S. hermonthica* in upland rice.

Materials and methods

Field trials were conducted in the dry (Experiment I) and wet (Experiment II) seasons of 1997 at Samaru (11°11'N, 7°38'E, 686 m above sea level) in the northern Guinea savanna ecological zone of Nigeria. The soil has a sandy loam texture. Five varieties of upland rice (FARO 46, FARO 11, FARO 45, FARO 48 and FARO 38) formed the main plot treatments, while five rates of nitrogen (0, 30, 60, 90 and 120 kg N/ha) constituted the subplot treatments. The trials were laid out in a split plot design with three replications. In the dry season, land preparation was accomplished after the experimental area was well soaked with pipe-borne water using garden hoses. In the wet season the land was mechanically ploughed, disc-harrowed and ridged. The individual plots were marked out and turned into basins to prevent the flow of the nitrogen fertilizer from one plot to another. The dry and wet season trials were planted on the 11th April and 29th June, respectively. The plant spacing was 25 × 25 cm. All the phosphorus and potassium, at a rate of 30 kg P₂O₅ and 30 kg K₂O, as well as half of the nitrogen were applied at 21 days after sowing (DAS) using 15–15–15 compound fertilizer followed by topdressing at the appropriate rates at 60 DAS.

To ensure the uniform distribution of *Striga* seeds in the soil, the fields were inoculated immediately before planting the rice by placing 3.0 g of inoculating materials in each planting hole, thus ensuring approximately 3000 germinable seeds/hill (Kim, 1994; Magani, 1994). In the dry season, all the plots were watered to field capacity with garden hoses during the first two months (April/May). Two hoe-weedings were carried out at 14 and 28 DAS. Thereafter, hand pulling of weeds of other species was employed till harvest in order to prevent removal of the emerging *Striga* plants. The data collected were: number of days to first *Striga* emergence, emerged *Striga* shoots (infestation), and crop reaction scores at 9 and 12 weeks after sowing (WAS) and at harvest. Crop reaction score was based on a 1–9 scale, where (1) was assigned to plots with healthy plants and (9) to those with completely dead plants (Kim, 1994). The number of days to 50% flowering, 1000-grain weight and grain yield of rice were also recorded. The number of days to 50% flowering of rice in the dry season trial was not reliable and was therefore discarded. All the data were subjected to analysis of variance to test significance and the treatment means were compared using the Duncan Multiple Range Test (DMRT) at the 5% level of probability.

Results

The upland rice varieties differed significantly in their support for *Striga* infestation in the wet season trials (Table 1). The application of 60 to 120 kg N/ha in the dry season trial and of 90 and 120 kg N/ha in the wet season significantly delayed *Striga* emergence on upland rice compared with 0 kg N/ha. The interaction of varieties of upland rice and nitrogen rates on the number of days to first *Striga* emergence was significant in the wet season trial (Table 2). The application of 120 kg N/ha caused the maximum delay in *Striga* emergence in FARO 11. Only FARO 48 and FARO 38 given 120 kg N/ha supported first *Striga* emergence at a number of days comparable to the maximum and more than the least.

The varieties of upland rice also differed significantly in their reaction scores to *Striga* at 9 weeks after sowing (WAS) and in grain yield in the two trials (Table 3). In both the trials FARO 48 consistently exhibited a lower reaction score than the appropriate maxima. While all the other varieties

exhibited similar reactions to *Striga* in the wet season, FARO 11 and FARO 38 also had lower scores than FARO 45 in the dry season trial. Nitrogen application at 60–120 kg N/ha in the dry season and at all rates in the wet season significantly reduced the crop reaction score to *Striga* infestation in upland rice compared with no nitrogen. In the wet season the crop reaction score of upland rice decreased with increasing levels of nitrogen up to 90 kg N/ha. The interaction of upland rice varieties and nitrogen rates on the crop reaction score was significant in the wet season trial. The maximum crop reaction score occurred for FARO 38 without nitrogen application (Table 4). Conversely, FARO 46 given 60 and 90 kg N/ha, FARO 11 given 90 kg N/ha, FARO 45 given 60 and 120 kg N/ha, FARO 48 given 90 kg N/ha and FARO 38 given 120 kg N/ha had crop reaction scores comparable to the least (FARO 11 given 120 kg N/ha).

FARO 11 produced grain yield that was maximum in the wet season and comparable to the maximum, produced by FARO 48, in the dry season trial. Contrarily, FARO 46 and FARO 45 in both trials and FARO 48 in the wet season trial produced significantly lower grain yield than FARO 11. Nitrogen application had a significant effect on upland rice grain yields in all the trials. The grain yield was significantly increased by the application of 90 and 120 kg N/ha in the dry season trial and increased with nitrogen levels in the wet season trial.

Table 1

Effect of nitrogen on the number of days to first *Striga* emergence and *Striga* shoot count in upland rice at Samaru in the dry (Experiment I) and wet (Experiment II) seasons of 1997

Treatments	No. of days to first <i>Striga</i> emergence		<i>Striga</i> shoot count at					
			9 WAS		12 WAS		Harvest	
	Expt I	Expt II	Expt I	Expt II	Expt I	Expt II	Expt I	Expt II
Varieties (V)								
FARO 46	69.9	55.6	3.0	3.8ab	3.5	10.9ab	6.3	20.1b
FARO 11	78.7	59.3	2.9	1.7b	3.1	4.1bc	6.3	9.7b
FARO 45	73.9	63.5	5.0	1.1b	5.1	3.7c	10.2	8.1b
FARO 48	75.2	61.5	3.5	1.1b	3.4	4.9bc	7.5	10.8b
FARO 38	75.0	57.7	4.0	5.0a	4.5	16.6a	9.4	30.9a
SED	6.16	3.09	3.27	1.84	2.74	4.55	3.59	5.75
kg N/ha (N)								
0	67.5b	54.9b	2.9	2.7	3.1	7.5	7.0	13.8
30	70.2ab	57.3ab	2.4	2.3	2.7	8.1	6.6	19.3
60	77.1a	57.0ab	3.9	4.1	4.1	11.7	8.5	21.3
90	79.1a	64.5a	3.8	1.7	4.1	6.1	8.1	13.7
120	78.9a	64.0a	5.3	1.7	5.5	6.8	9.5	10.9
SED	4.23	3.75	1.37	1.51	1.33	3.28	1.88	6.41
V×N	NS	*	NS	NS	NS	NS	NS	NS

Means followed by the same letter(s) are not significantly different at the 5% level of probability (DMRT); NS = non-significant

Table 2

Interaction of upland rice varieties and nitrogen on number of days to first *Striga* emergence on upland rice at Samaru in the wet season of 1997

Varieties	Nitrogen (kg/ha)				
	0	30	60	90	120
FARO 46	60.3abc	58.3abc	54.3bc	58.7abc	46.3c
FARO 11	57.3bc	56.7bc	55.7bc	55.0bc	77.7a
FARO 45	53.0bc	55.7bc	54.0bc	55.3bc	60.0bc
FARO 48	59.7abc	61.0abc	57.0bc	56.7bc	73.3ab
FARO 38	58.0bc	54.7bc	53.3bc	59.3abc	62.7abc
SED			8.39		

Means followed by the same letter(s) are not significantly different at the 5% level of probability (DMRT)

Table 3

Effect of nitrogen on the crop reaction score, number of days to 50% flowering, 1000-grain weight and grain yield of upland rice under *Striga hermonthica* infestation at Samaru in the dry (Experiment I) and wet (Experiment II) seasons of 1997

Treatments	Crop reaction score at 9 WAS		Days to 50% flowering	1000 grain weight (g)		Grain yield kg/ha	
	Expt. I	Expt. II	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II
Varieties (V)							
FARO 46	3.9ab	5.4a	85.1d	36.9a	34.9a	1643c	241c
FARO 11	2.7cd	5.1a	99.6b	34.7ab	30.8b	2469ab	374a
FARO 45	4.4a	5.0a	82.1e	32.9b	31.7b	1773c	216c
FARO 48	2.1d	4.1b	107.4a	32.2b	31.0b	2772a	186c
FARO 38	3.5bc	5.5a	87.9c	37.0a	35.2a	2170bc	315b
SED	1.49	0.30	0.74	0.20	0.99	259.5	42.7
kg N/ha (N)							
0	4.7a	6.7a	93.2a	33.3	31.2b	1635b	103e
30	4.1a	5.7b	92.3ab	34.5	32.9ab	2117ab	188d
60	2.9b	5.1c	92.2ab	35.8	33.8a	2164ab	261c
90	2.5b	4.4d	91.9b	34.6	32.6ab	2383a	358b
120	2.5b	4.3d	92.5ab	35.6	33.1ab	2528a	422a
SED	0.38	0.22	0.49	0.13	1.13	260.4	28.76
V×N	NS	*	NS	NS	NS	NS	NS

Means followed by the same letter(s) within a column are not significantly different at the 5% level of probability (DMRT); NS = non-significant

Table 4

Interaction of upland rice varieties and nitrogen on crop reaction score at 9 WAS at Samaru in the wet season of 1997

Varieties	Nitrogen (kg/ha)				
	0	30	60	90	120
FARO 46	6.7ab	6.7ab	4.7d-g	4.0fg	5.0c-f
FARO 11	6.7ab	5.7bcd	5.3cde	4.0fg	3.7g
FARO 45	6.7ab	5.3cde	4.3efg	5.0c-f	4.7d-g
FARO 48	6.7ab	5.3cde	5.0c-f	4.0fg	4.3efg
FARO 38	7.0a	5.7bcd	6.0abc	5.0c-f	4.0fg
SED			0.48		

Means followed by the same letter(s) are not significantly different at the 5% level of probability (DMRT)

Discussion

In the study FARO 48 exhibited enhanced resistance through support for delayed and low *Striga* emergence, low crop reaction score and high grain yield, while FARO 11 exhibited tolerance. This variety produced high grain yield in spite of support for early and high *Striga* incidence. Several results earlier showed that nitrogen enhances crop tolerance to the parasite (Babiker, 1991; Adetimirin et al., 1997; Kim and Adetimirin, 1997). Ransom et al. (1996) also indicated that the superior grain yields of resistant varieties of maize compared with susceptible varieties was related to delayed or no *Striga* parasitism on the resistant varieties. Similarly, Johnson et al. (1997) confirmed that resistant cultivars of rice exhibited no or lower levels of parasitism by *Striga* compared with susceptible cultivars. Furthermore, Ejeta et al. (1999) also indicated that *Striga*-tolerant genotypes permit and support as many *Striga* plants as susceptible genotypes but do not show a concomitant reduction in grain production or overall productivity. FARO 38, FARO 46 and FARO 45 exhibited susceptibility to *Striga hermonthica* even with the application of nitrogen. Riches et al. (1996) reported that susceptible rice varieties produced stunted plants which did not flower at all and that little biomass was harvested compared with resistant varieties. The grain yields of rice obtained in the dry season were higher than those obtained in the wet season. The intensive precipitation and consequent excess soil moisture that occurred in the months of August and September probably caused leaching and reduction of the applied nitrogen in the wet season.

Conclusions

Among the varieties tested, FARO 48 exhibited enhanced resistance in this study. FARO 11 exhibited tolerance, while FARO 38, FARO 46 and FARO 45 exhibited susceptibility. In the study, the application of 90 kg N/ha consistently delayed and reduced *Striga* emergence on upland rice and caused low crop reaction scores compared with no nitrogen, thus resulting in increased grain yield compared to the maximum obtained with 120 kg N/ha. The lower rates of nitrogen, which caused a similar reduction in *Striga* incidence, resulted in significantly lower yields than the application of 90 or 120 kg N/ha.

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CHEMICAL COMPOSITION OF BERMUDA GRASS (*CYNODON DACTYLON*) IN HUNGARY

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Under the influence of the prognosticated climatic changes, the increasing rate of degradation and the extension of uncultivated lands, it is expected that the dominance of some C₄ plants will increase. Bermuda grass (*Cynodon dactylon*) is the most common C₄ species in Hungary. The chemical composition of *Cynodon dactylon* and its substrate was investigated on 3 soil types (Arenosol, Solonchak soil and a waste place) typical of the country. It was established that in comparison with other perennial C₄ grasses (*Andropogon ischaemum*, *Chrysopogon gryllus*, *Cleistogenes serotina*) the total element content of *Cynodon dactylon* was the highest. A detailed quantitative and qualitative knowledge of the chemical components of C₄ plants could help to determine the expected changes in the chemical composition of the uppermost soil layer and in its mineralization dynamics on areas dominated by these plants. As a result of the expansion of *Cynodon dactylon* the element concentration and the chemical composition of the soils might change, thus influencing successional processes as well.

Key words: Arenosol, Bermuda grass, chemical composition, *Cynodon dactylon*, Hungary, Solonchak soil, waste place

Introduction

According to previous surveys (Kalapos, 1991; Kalapos et al., 1997) about 60 C₄ species occur in Hungary. The majority of these belong to the grass family (*Poaceae*). Five of them (*Andropogon ischaemum*, *Chrysopogon gryllus*, *Cleistogenes serotina*, *Cynodon dactylon* and *Sorghum halepense*) are perennials, the last of them having become naturalized in Hungary during the last few decades. *Cynodon dactylon* is the most common of these species.

Under the influence of the prognosticated climatic changes (deduced from the lower rainfall registered during the last few decades and the above-average temperatures during the last few summers), the increasing rate of degradation and the extension of uncultivated lands, the dominance of some C₄ plants is expected to increase. Another reason for the extension of synanthropic areas may be the increasing nutrient content of the soils (eutrophication) and the expansion of the sodic-saline areas (Hoffmann, 1994).

The cosmopolitan species Bermuda grass prefers the tropical, subtropical and temperate climatic zones (Hakansson, 1995). Its expansion strategy is based

on its aboveground stolons and underground rhizomes. Particularly in devastated areas, devoid of other competitor species, *Cynodon dactylon* can overgrow a 13–25 m² area within 1.5–2 years (Horowitz, 1972; 1996). In Western Europe *Cynodon dactylon* is spreading mainly in disturbed areas, whereas in Hungary perennial C₄ grasses (*Botriochloa*, *Chrysopogon*, *Cleistogenes*, *Cynodon*) are spreading on native grasslands dominated by *Festuca vaginata*, *F. pallescens*, *F. rupicola* and *F. valesiaca*, thus changing their species composition (Virágh and Fekete, 1984; Tóth, 1988; Zólyomi and Fekete, 1994; Bagi, 1997).

A detailed quantitative and qualitative analysis of the chemical components of C₄ plants could help to determine the changes to be expected in the chemical composition of the upper soil layer and in its mineralization dynamics. The present paper provides information on the chemical composition of *Cynodon dactylon*, a species widely distributed in Hungary.

Materials and methods

Due to its rhizome system, the invasion potential of *Cynodon dactylon* is higher than that of *Festuca* species, which have fibrous root systems. Besides vegetative reproduction its competitive abilities are also increased by its generative reproduction as well as by its drought tolerance, which is a general feature of C₄ plants. This eurytopic species is common on the following soil types: Leptosols on limestone, dolomite or volcanic rocks, loess (chernozems), Arenosols, solonchak and solonetz soils, waste places and uncultivated areas.

The chemical composition of *Cynodon dactylon* and its substrate was investigated on 3 soil types: Arenosol, solonchak soil and a waste place. Five sampling sites were selected on each soil type. At each sampling site 10–15 plants were collected together with their supporting soils to a depth of 20 cm. The samples were collected at the end of July or at the beginning of August.

The soil samples were dried to constant weight. The plants were washed with distilled water, and their roots, stems and leaves were separated. The organs originating from 10–15 plants were amalgamated before digestion with nitric acid and hydrogen peroxide under pressure. The samples were then analysed for the following 26 elements: Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Si, Sr, Ti, V and Zn, using an ICP-AES at the Department of Chemistry of the Horticultural Faculty of Szent István University. The C and N contents of the samples were also analysed. The total nitrogen content was determined following digestion with sulphuric acid (Kjeldahl method) using a Contiflo analyser. The carbon content was measured, after dry combustion using the method of Dumas, by gas chromatography with an NA-1500 analyser produced by Fisons. In the present work the chemical composition of the leaves was used for comparative evaluations, because this plant organ accumulates the highest amounts of elements.

Results

The N content of the different plant parts was relatively low, whereas the C concentration was relatively high (Table 1).

Table 1
C and N contents (in %) and C/N ratio of the organs of *Cynodon dactylon* (n: 5)

Sample	Soil type	Arenosol			Solonchak soil			Waste place		
		N	C	C/N	N	C	C/N	N	C	C/N
Leaf		1.43	39.98	27.8	1.52	39.18	25.68	1.89	39.83	21.07
Stem/stolon		1.15	41.77	36.2	1.14	43.77	38.33	–	–	–
Root		1.43	36.37	25.3	1.1	44.08	40	1.38	40.46	29.31

The total element content of the leaves of *Cynodon dactylon* is usually between 21,000 and 24,000 $\mu\text{g g}^{-1}$ (Table 2). It seems that, irrespective of the soil type, Bermuda grass accumulates a large amount of potassium (Table 3). The leaves of plants on sandy soils in the Tisza-Danube region chiefly accumulated Ca and Mg, while the corresponding organs of plants growing on saline soils accumulated Na, and those in ruderal areas accumulated P. The various organs of Bermuda grass contained different amounts of the ions examined. The concentration of ions was highest in the leaves (Table 2). Samples from alkaline soils and ruderal areas accumulated more elements in their stems/stolons than in their roots. In contrast, in plants growing on sandy soils the element concentration of the roots was higher than that of the stems/stolons. The C concentration in the organs of Bermuda grass was relatively high, whereas the N concentration was rather low except in plants growing on waste places (Table 1).

Table 2
Total element content of the organs and soils of *Cynodon dactylon* ($\mu\text{g g}^{-1}$) (n: 5)

Sample/Soil type	Arenosol	Solonchak soil	Waste place
Soil	27 122	81 091	34 839
Leaf	21 892	22 829	23 285
Stem/stolon	10 269	13 814	14 257
Root	16 877	9037	13 372

Discussion

In comparison with other perennial C_4 grasses (*Andropogon ischaemum*, *Chrysopogon gryllus* and *Cleistogenes serotina*) the total element content of *Cynodon dactylon* is the highest. It is only exceeded by *Sorghum halepense*, which is an adventive species growing on habitats with anthropogenic impacts (arable and uncultivated lands as well as waste places). A special feature of Bermuda grass is that it takes up a specific quantity of ions, largely independently of the element content of the soil.

Albert (1982) also established that halophytes concentrate more ions in their roots than in their leaves. A high K concentration is usually typical of grass species (Kinzel, 1982). In comparison with other Hungarian perennial grasses the largest amount of potassium is concentrated in the leaves of Bermuda grass.

The species takes up a large amount of potassium even from soils containing only a small quantity of this element (e.g. from the sandy soils of the Danube-Tisza region, see Table 3). The concentration factor (element content of the plant/element content of the soil) of K is approximately 68. According to the investigations of Utrillas et al. (1995), the high K content of the leaves during the summer months contributes to higher osmotic pressure. An increase in the K concentration facilitates the better adaptation of the plants to water stress. Due to the higher K concentration the transpiration intensity is lower (Pethő, 1984). This ecophysiological feature is characteristic of C_4 grasses. The higher K concentration increases frost resistance, thus also contributing to the higher competitiveness of *Cynodon dactylon*. Besides some morphological features (the presence of stolons and rhizomes in the shoot system of the plant) the higher K content of the leaves may also promote adaptation to extremely dry conditions. Besides the high K content the leaves also contain a relatively large amount of Ca. On the basis of the K/Ca ratio *Andropogon ischaemum*, *Chrysopogon gryllus* and *Cleistogenes serotina* belong to the calciotrophic physiotype, and on the basis of this ratio (1.78–2.61) *Cynodon dactylon* is also close to this physiotype.

Bermuda grass is also common on saline soils. On solonchak soils the Na concentration of the leaves often exceeds $1800 \mu\text{g g}^{-1}$. The K/Na ratio (6–4) measured in the leaves is characteristic of halophytes. According to Albert (1982) *Cynodon dactylon* is a salt-tolerant, facultative halophyte.

C_4 species require Na in micro concentrations (Pethő, 1984). In the present investigations perennial C_4 grasses had a higher Na content than C_3 species growing on the same habitats.

The relatively high C concentration (Table 1) seems to be the consequence of the intensive CO_2 uptake. With the exception of plants growing in ruderal habitats the C/N ratio exceeded the critical value determined for decomposition. This results in slower mineralization dynamics. Considering the expansion of *Cynodon dactylon* into dry areas the following consequences can be forecasted:

- Due to the C/N ratio of the different plant parts and the slower rate of decomposition, the organic material content and the adsorption capacity of the soil will increase.
- The total element concentration of the uppermost soil layer will increase and its chemical composition will change as a consequence of K, Ca and P accumulation.
- The increasing nutrient content of the soils will be favourable for the spread of C_4 species.
- The increasing element concentration and the changed chemical composition of the soils may have some influence on the process of succession.
- The change in the total element concentration of the uppermost soil layer may influence the floristic composition of dry grasslands (sand steppes, steppic grasslands).

Table 3

Element contents ($\mu\text{g g}^{-1}$ dry weight) of the leaves of *Cynodon dactylon* samples and of three typical soil types supporting their growth

Elements	1		2		3	
	Soil (0–20 cm)	Leaf	Soil (0–20 cm)	Leaf	Soil (0–20 cm)	Leaf
Al	3128	267	477	325	2070	173
As	<d.l.	<d.l.	0.04	<d.l.	<d.l.	<d.l.
B	8.72	45.1	2.66	19.2	2.43	4.20
Ba	47.1	12.1	15.7	12.5	42.7	15.3
Ca	52031	6262	21964	5640	23277	5006
Cd	1.35	0.13	0.17	0.16	0.89	0.13
Co	2.61	0.13	0.64	0.20	2.13	0.13
Cr	5.63	0.91	0.19	1.13	4.01	2.22
Cu	7.28	6.97	2.95	7.81	20.0	8.74
Fe	5029	120	281	421	3413	301
Ga	3.60	0.25	0.73	0.66	2.70	0.70
K	756	11179	164	8945	581	13097
Li	5.77	17.6	0.35	6.83	2.89	0.56
Mg	17867	1736	3458	1924	4035	1622
Mn	200	51.8	115	46.5	196	44.3
Mo	<d.l.	0.36	<d.l.	0.10	<d.l.	0.23
Na	837	164	37.3	1519	77.8	374.80
Ni	7.92	3.93	1.44	2.05	6.15	2.47
P	427	1255	298	1336	406	2106
Pb	6.32	1.21	6.26	2.05	18.1	1.57
Se	<d.l.	0.41	<d.l.	<d.l.	<d.l.	<d.l.
Si	497	735	252	587	579	456
Sr	188	24.7	35.5	31.8	43.1	22.6
Ti	39.5	2.37	2.30	6.00	18.8	4.34
V	4.73	0.11	1.06	0.46	4.51	0.28
Zn	19.4	18.4	6.08	41.9	35.4	47.4

1: Tisza-Danube region (Tatárszentgyörgy, Ladánybene, Kecskemét, Izsák-Orgovány, Szabadszállás), Arenosol (n: 5)

2: Szabadszállás I-II, Apaj-pusztá, Kunszentmiklós, Sárkeresztúr, solonchak soil (n: 5)

3: Budapest I-II, Gödöllő, Órbottyán, Nagykáta, waste place (n: 5)

<d.l.: values under the detection limit

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VARIATIONS IN GERMINATION AND LONGEVITY OF PEPPER (*CAPSICUM ANNUUM* L.) SEEDS HARVESTED AT DIFFERENT STAGES OF MATURATION

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Pepper (*Capsicum annum* L.) seeds of the cultivars Tatashe and Rodo, extracted from fruits sequentially harvested at different maturation stages, were tested for germinability (when freshly harvested) and longevity (over a period of about 17 weeks). Seed dry weight increased with fruit maturation and was maximum when the fruits turned red-ripe. Over-ripening of the fruits for 10 days on the mother plant did not result in a significant seed weight increase, signifying that the seeds were already fully filled when the fruits turned red-ripe. The seeds of the two cultivars started germinating as from 28 days after anthesis (DAA) and peaked at 71.5% and 40% for Tatashe and Rodo respectively, at 44 DAA. The viability of the seeds of Tatashe declined as storage progressed and the more mature the seeds were, the longer they survived. In Rodo, seed viability increased till the end of the storage period due to dormancy, which became progressively broken with age; seeds from more mature fruits survived better. In a second experiment, when fruits of Tatashe were harvested at the colour breaking, red-ripe and over-ripe stages, seed viability and longevity were best from over-ripe fruits.

Key words: pepper, *Capsicum annum*, germination, maturation, longevity, different stages

Introduction

The decision on when to harvest a seed crop is influenced amongst other things by the state of fruit maturity. When pepper is being harvested for both fruits and seeds, the fruits are harvested at various degrees of ripeness. In the fruit trade, fully ripe fruits are sold in local markets while less ripe ones are reserved for distant markets as they would normally ripen in transit. For the seed crop, all the fruits at various stages of ripening are normally processed at once and the seeds from them bulked into one lot. This practice arises from the assumption that pepper seeds are mature, and would therefore give maximum quality, as soon as the fruit colour starts changing from green. Contrary to this belief, Belletti and Quagliotti (1991) reported that the highest percentage of seed germination can be obtained from fully (red or yellow) coloured fruits. This report is supported by recent findings (unpublished) in our laboratory in which seeds extracted from fully ripe fruits of the cultivars Rodo and Tatashe germinated significantly better than those from less ripe ones. Jacobsen et al. (1999) reported that the percentage germination improved at later harvesting dates in quinoa. However, whereas in some species the highest quality is

normally obtained at the end of the seed-filling period (Harrington, 1972; Rasyad et al., 1990), maximum quality might not be obtained until some time after this in others (Kameswara et al., 1991). Furthermore, delayed harvesting has been reported to reduce seed quality in some other crops (Singh et al., 1976; Nkang and Umoh, 1996; Valdes and Gray, 1998). Louwaars and Marrewijk (1996) listed harvesting seeds at the optimum stage of seed development as one of the important preconditions for a good quality seed supply.

The longevity of a pepper seed sample is known to depend, among other things, on the initial vigour of the sample (Belletti and Quagliotti, 1991). McAlister (1943) reported that the superiority of mature seeds of *Agropyron* and *Elymus* over those harvested earlier than the dough stage, became especially noticeable after storage. Similarly, the result of a study conducted on *Amaranthus paniculatus* revealed that black seeds, which are normally assumed to be more mature than brown ones, survived longer than the latter, though viability levels were identical prior to storage (Oladiran and Mumford, 1989). Nkang and Umoh (1996) also reported that soybean seeds harvested at agronomic maturity survived longer than those harvested at physiological maturity, two weeks after agronomic maturity, despite the fact that the germination percentage for all cultivars was quite high before the seeds were stored.

The present study was undertaken to determine how seed viability and vigour varied with stage of development, in order to be able to recommend the appropriate stage to harvest pepper fruits for optimum seed quality both before and after storage.

Materials and methods

Pepper crops of cultivars Rodo and Tatashe were raised in the field during the rainy season of 2000. Individual flowers were date-tagged as they opened (immediately after anthesis). Fruits that developed from the tagged flowers were harvested at intervals of four days starting at eight days after anthesis (DAA). Harvesting was terminated at 44 DAA. At each harvest, the seeds were immediately extracted from the fruits and dried to constant weight at room temperature, following which the dry seed weight was determined.

In another study, fruits of Tatashe were tagged at the colour breaking stage. Some of these fruits were harvested on the same day they were tagged (CB), while others were harvested when they became fully ripe (FR). The rest of the fruits were harvested 10 days later (OR). Following seed extraction, data were collected on 100-seed weight and germination percentage. This study was conducted to ascertain the changes that occur in seed vigour after the colour breaking (CB) stage on the mother plant.

Seed samples from both experiments were packaged in paper envelopes and stored in an ambient environment (approx. 30°C and 40% relative humidity). In Tatashe, germinability was tested after 6 and 17 weeks of storage, while in Rodo the seeds were sampled for germination at 5 and 16 weeks. Germination tests, made immediately before and during storage, were conducted on four replicates of 50 seeds each, spread over distilled water-moistened absorbent paper in Petri dishes and incubated at 30°C for 28 days. Counts were taken every other day.

Results

By about 40 DAA, the fruits were greenish red and turned fully red by 44 DAA. Figure 1 shows that the dry seed weight per fruit increased with fruit age in both cultivars, with higher values in Tatashe, and was highest in both cultivars by 44 DAA.

In both cultivars, germination was not recorded earlier than 28 DAA in freshly harvested seeds (Figs. 2 and 3). Thereafter, germinability increased to a maximum of about 71% and 40% in Tatashe and Rodo, respectively, by 44 DAA. Following seed storage, viability declined with age in all seedlots of Tatashe, with seeds extracted from fruits harvested at 44 DAA storing better than those from less mature fruits (Fig. 2). In contrast to the behaviour of Tatashe seeds, Figure 3 reveals that germination improved with storage time in Rodo seeds, so that a germination percentage of about 75% was recorded after 16 weeks' storage as against 40% in unaged (fresh) seeds from fruits harvested at 44 DAA. Similarly, seeds harvested at 40 DAA produced a germination percentage of about 61% after 16 weeks' storage compared to about 20% when freshly harvested. This trend indicated the presence of dormancy in the freshly harvested Rodo seeds. The 100-seed weights of Tatashe seeds extracted from fruits harvested at the colour breaking (CB), fully ripe (FR) and over-ripe (OR) stages were 0.35 g, 0.44 g and 0.45 g, respectively. Statistical analysis revealed that though there was no significant ($P=0.05$) difference between FR and OR seeds, they were both significantly heavier than the CB seeds. Figure 4 shows the germination of fresh and stored CB, FR and OR seeds of Tatashe. When freshly harvested, germination was significantly ($P=0.05$) higher from OR seeds than from FR seeds, which in turn had significantly ($P=0.05$) better germination than CB seeds. Following 6 and 17 weeks of storage, seed viability declined progressively, though OR still maintained superiority over the other two seedlots and CB seeds were still the poorest in quality.

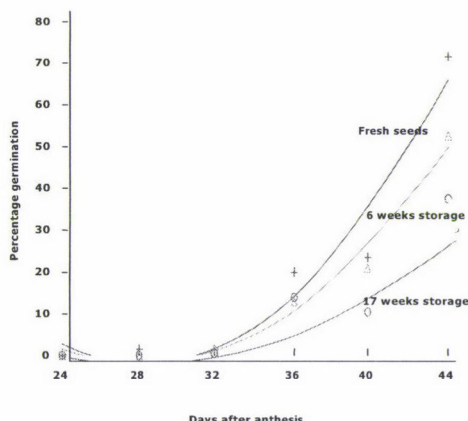
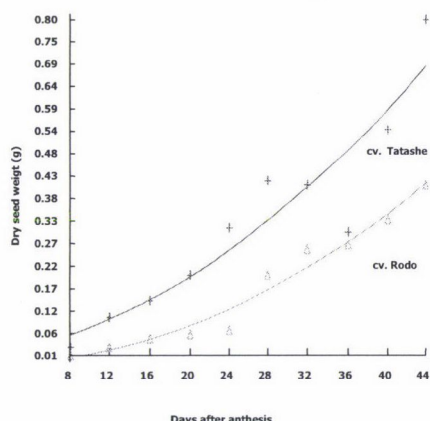


Fig. 1. Dry seed weight (g)/fruit in cvs. Tatashe and Rodo at different stages of maturation

Fig. 2. Germination of fresh and stored Tatashe seeds extracted from fruits at different maturation stages

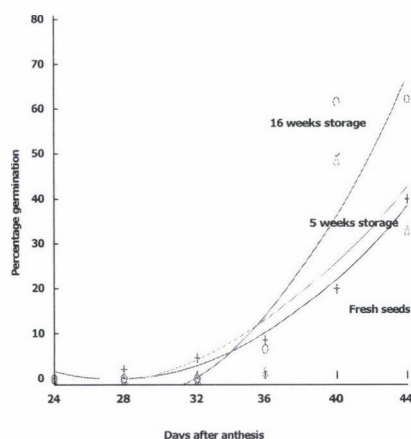


Fig. 3. Germination of fresh and stored Rodo seeds extracted from fruits at different maturation stages

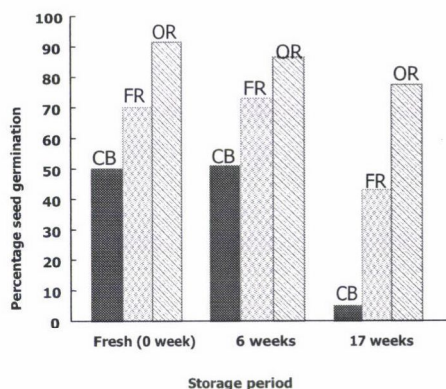


Fig. 4. Germination of fresh and stored seeds of cv. Tatashe extracted from fruits at colour breaking (CB), fully ripe (FR) and over-ripe (OR) stages

Discussion

The highest dry seed weight for the two cultivars used in this study was obtained at 44 DAA. The lack of a significant difference in the 100-seed weights of Tatashe seeds extracted from fully ripe and over-ripe fruits suggests that there will be no further significant changes in seed weight. The red-ripe stage could therefore be taken as the end of the seed-filling period. Since the highest seed germination percentage and better longevity were obtained at this stage when sequential harvesting was done, it could be assumed that maximum seed quality was already attained at the red-ripe phase, which agrees with the reports of Belletti and Quagliotti (1991) and Oladiran and Haruna (2001). However, the results of the present study revealed that when Tatashe fruits were allowed to over-ripen on the mother-plant, greater seed vigour (indexed by percentage germination and longevity) was obtained. Ellis et al. (1987) reported that the maximum seed quality of some grain legumes occurred when the seeds had maximum percentage germination. To obtain maximum seed quality, fruits may have to be allowed to over-ripen on the mother-plant before they are harvested. Kameswara Rao et al. (1991) reported that maximum seed quality was not obtained in pearl millet until some time after the end of the seed-filling period. A similar observation was reported for rice by Ellis and Jackson (1995).

The general decline in the viability of Tatashe seeds in storage is in line with the expected trend, though the improvement in Rodo seed germination as storage progressed is not uncommon in pepper either. Recently, Oladiran and Agunbiade (2000) reported that within the first six weeks of storage, aged

pepper seeds germinated faster than freshly harvested ones and that the seedlings produced from aged seeds were taller and heavier than those from unaged ones. This was attributed to the existence of dormancy in freshly harvested pepper seed, which is normally depleted during after-ripening (Randle and Honma, 1981). However, whereas dormancy was broken within six weeks of storage in the work reported by Oladiran and Agunbiade (2000), in the present study Rodo seeds still gave improved germination following 16 weeks' storage compared to the values obtained after five weeks of storage. This suggests that different seedlots of the same cultivars or varieties may require different after-ripening periods.

It is concluded that pepper seed may not attain maximum quality until the fruits over-ripen. It is therefore recommended that pepper fruits be harvested at the over-ripened phase, but they should not be allowed to rot, as other work such as that reported by Valdes and Gray (1998) has shown that delayed harvest led to deterioration of the seeds in tomato. As an alternative, fruits may be harvested when fully ripe and then kept aside to after-ripen for some days, as this was shown by Quagliotti et al. (1981) to result in a noticeable improvement in germination.

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EVALUATION OF BORON FERTILIZERS FOR A SUNFLOWER (*Helianthus annuus* L.) – GREEN GRAM (*Vigna radiata* L.) CROPPING SEQUENCE IN INCEPTISOLS

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Field experiments were conducted at the Agricultural Research Station, Bhavanisagar, Tamil Nadu, India during 1999 to 2000 in Inceptisols to evaluate boron fertilizers (borax, boric acid, Agribor) and to assess the effect of the mode and level of B application on the total B uptake and yield of sunflower and green gram. The experimental field was found to be deficient in available B (0.35 mg kg^{-1}). Sunflower was raised as the main crop. Different B levels (0.5, 1.0, 1.5 and 2.0 kg B ha^{-1}) as soil application and two levels of foliar spray (0.2% and 0.3%) were compared with the control. The treatments were superimposed on the recommended dose of NPK. After harvesting the main crop of sunflower, the residual effect of B was studied by raising green gram on the same field. The results revealed that the application of all the levels of B resulted in a significant increase in the B uptake and yield of sunflower as compared to the control. The highest yield was recorded for the soil application of B at a rate of 2.0 kg ha^{-1} . The yield increase of sunflower was 3.6 to 15.8% and 7.2 to 18.9% over the control for seed and stalk, respectively. The green gram grain yield increased by 4.2 to 13.5% after the application of 1.0 and 2.0 kg B ha^{-1} to the main crop. No residual effect was observed for the lowest level of B application (0.5 kg ha^{-1}). Agribor is equally as effective as borax in influencing the B nutrition of both the crops.

Key words: boron nutrition, total B uptake, yield, sunflower, residual effect, green gram

Introduction

Sunflower is the second most important edible oilseed in the world next to soybean. In India it occupies nearly 11% of the total oil seed cropped area. It has been proved to be highly promising in the different agro-climatic regions of India because of its thermoinsensitivity and high production potential. However, the productivity of the crop is low. Sunflower is the most sensitive field crop to low B supply and in B-deficient sunflower plants there is an acceleration of protein decomposition and a retardation of protein synthesis. B deficiency in this crop has been reported all around the world, and is found in Tamil Nadu, India in the coarse-textured soils (Krishnasamy et al., 1994). In order to increase productivity, balanced fertilization with micronutrients is essential.

B is essential for the growth and development of plants. The importance of boron as an essential micronutrient for the maintenance of structural integrity, the germination of pollen grains, the development of pollen tubes and seed filling, has been emphasized by many research workers (Narkhede and Patil, 1989; Ismail and Volker, 1997). Hence a study was undertaken to find the effect of different sources, methods and levels of B application on the yield of sunflower as main crop and of green gram as residual crop.

Materials and methods

Field experiments were conducted at the Agricultural Research Station, Bhavanisagar, Tamil Nadu Agricultural University during 1999 to 2000 in Inceptisols of the Typic Ustropept type. The station is situated at 11° 29' N and 77° 8' E at an altitude of 256 m above sea level in Tamil Nadu, India.

The soil type of the experimental field was a red, non-calcareous Inceptisol (Typic Ustropepts). The pH of the soil was neutral (7.2) and the electrical conductivity 'Non saline' (0.34 EC dSm⁻¹). The initial soil sample was analysed for the available B, N, P and K using Azomethine H reagent, alkaline permanganate, 0.5 M NaHCO₃ and neutral normal ammonium acetate, respectively. The analysis showed the experimental field to be deficient in available B (0.35 mg kg⁻¹), low in available nitrogen (270 kg ha⁻¹) and medium in both available phosphorus (20.5 kg ha⁻¹) and potassium (220 kg ha⁻¹). The experiment was conducted in a factorial randomized block design replicated thrice. Two sources of boron, conventionally used fertilizers that are commonly used for soil application (borax and boric acid) (S₁) and Agribor (S₂) a new source, were used. The composition and properties of Agribor (S₂) are as follows:

Chemical formula	Na ₂ B ₁₀ O ₁₆ ·10 H ₂ O
Chemical name	Sodium decaborate decahydrate
pH	6.5–7.5 at 3% solution
Solubility	100 % soluble
Suitability	Both for soil and foliar application
Specific gravity	400 kg m ⁻³
Manufacturer	M/S BORAX MORAJI LIMITED

There were seven treatments comprising four levels (T₂–T₅) of B application in the soil (0.5, 1.0, 1.5 and 2.0 kg ha⁻¹) and two levels (T₆ and T₇) of foliar spray (0.2 and 0.3%), besides the check (T₁). The treatments were superimposed on the recommended dose of NPK.

The treatment details are given below:

T ₁	NPK alone
T ₂	NPK + boron 0.5 kg ha ⁻¹
T ₃	NPK + boron 1.0 kg ha ⁻¹
T ₄	NPK + boron 1.5 kg ha ⁻¹
T ₅	NPK + boron 2.0 kg ha ⁻¹
T ₆	NPK + foliar spray 0.2 % boric acid /Agribor*
T ₇	NPK + foliar spray 0.3 % boric acid /Agribor*

* Spray solution was prepared with a B content equivalent to that of 0.2% and 0.3% boric acid.

The sunflower cultivar Morden was raised as an irrigated crop. The soil applications were carried out by placing the B fertilizer along the furrows and the treatments were imposed before sowing. Foliar sprays were given three times during crop growth. The first spray was given on the 30th day and subsequent sprays at 10-day intervals. The residual crop green gram was sown after harvesting the main sunflower crop by keeping the layout the same. The yields of sunflower and green gram were recorded at maturity. Plant samples comprising seed and stalk (sunflower), or grain and stover (green gram) were analysed for B concentration (Banuelos et al., 1992). The experimental data were statistically analysed.

Results and discussion

Main crop: Sunflower

Total B uptake

Boron fertilization enhanced the concentration and uptake of B in the seed and stalk and an increase in total B uptake was also seen. Due to the increased

available B content in the soil, the soil application of Agribor at a rate of 2.0 kg ha⁻¹ led to the highest B content in the seed and stalk, followed by the 1.5 kg ha⁻¹, 1.0 kg ha⁻¹ and 0.5 kg ha⁻¹ applications. The foliar treatment also resulted in higher values and was found to be superior to the control (Fig. 1). The 2.0 kg ha⁻¹ application gave a total B uptake of 282 g ha⁻¹ compared to 108 g ha⁻¹ in the control. Both the sources were equally effective in influencing the total uptake of boron. Positive significant correlations were obtained between available B and plant B content at the vegetative ($r = 0.788$) and flowering ($r = 0.950^{**}$) stages and between plant B content and uptake at the vegetative ($r = 0.994^{**}$), flowering (0.986^{**}) and harvest ($r = 0.991^{**}$) stages. Similar observations were made by Shukla et al. (1983) in rai and by Ateeque and Malewar (1992) in sunflower.

Seed and stalk yield

The results revealed that the soil application of boron fertilizer increased the sunflower seed yield by 2.6 and 15.8% over the control at 0.5 and 2.0 kg ha⁻¹, respectively. For stalk yield, the increase was 7.2 and 18.9% respectively, for the same levels of boron. The 0.3% foliar spray (T₇) was on par with the soil application of B at 1.5 kg ha⁻¹ (T₄) for both seed and stalk yield. The influence of the sources for each treatment differed. It was observed that at a soil application level of 1.0 kg ha⁻¹ borax was better than Agribor for seed yield, while at 1.5 and 2.0 kg ha⁻¹ the reverse was true. The stalk yield was increased with increasing levels of B application and Agribor was found to be superior when compared to borax (Table 1). In the foliar spray treatments both the sources were equally effective irrespective of the concentration. Increased seed yield due to boron application was reported in groundnut by Patil et al. (1983). It was reported by Sutaria and Golakiya (1990) that B enhanced the yield-promoting attributes thus boosting the crop yield.

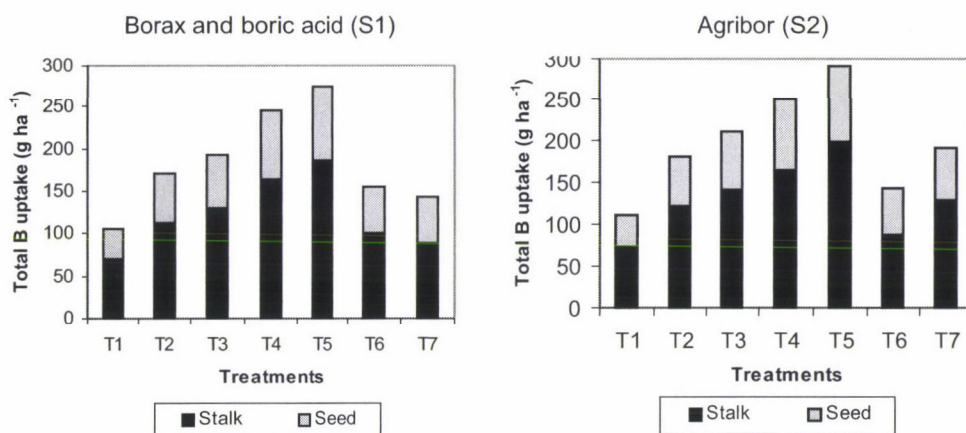


Fig. 1. Effect of sources, levels and methods of B application on the uptake of B in sunflower stalk and seed

Table 1
Effect of sources, levels and methods of B application on the seed and stalk yield
(kg ha⁻¹) of sunflower

Treatments	Seed yield			Stalk yield		
	S ₁	S ₂	Mean	S ₁	S ₂	Mean
T ₁	1642	1644	1643	3350	3355	3353
T ₂	1708	1704	1707	3604	3624	3614
T ₃	1803	1784	1794	3716	3844	3780
T ₄	1845	1909	1878	3844	4049	3947
T ₅	1914	1970	1943	3955	4320	4138
T ₆	1752	1783	1768	3664	3866	3765
T ₇	1870	1898	1884	3819	4033	3926
Mean	1791	1814	1803	3707	3870	3789
CD (P=0.05)	T = 29.13	S = 15.57	T×S = 41.19	T = 36.9	S = 19.7	T×S = 52.1

Residual crop: Green gram

Total B uptake

A concomitant increase in the residual boron was observed with an increase in the B levels applied. The highest boron uptake (125.0 g ha⁻¹) was observed in the treatment which received 2.0 kg ha⁻¹. The interaction between the treatment and source showed that there was no difference between the sources at lower levels of B application, while at higher levels (1.5, 2.0 kg B ha⁻¹) Agribor proved superior, registering an uptake of 123.7 and 131.0 g ha⁻¹ when compared to borax (113.9, 120.3 g ha⁻¹, respectively) for the same levels of boron. There was no residual effect of B to the plots which received the foliar spray treatments (Fig. 2).

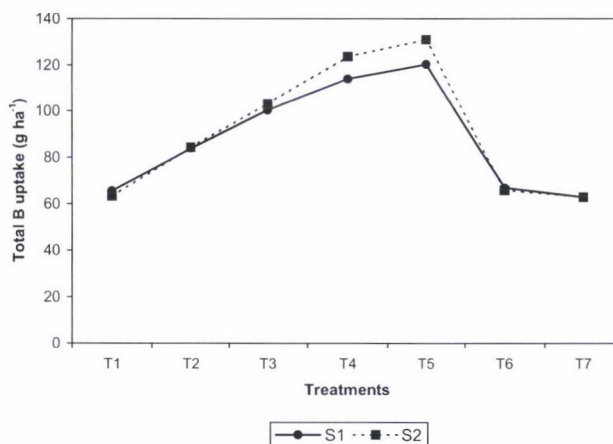


Fig. 2. Residual effect of sources and levels of B application on the total B uptake of green gram

Grain and stover yield

The highest grain and stover yields of 958 kg ha⁻¹ and 2662 kg ha⁻¹, respectively, were recorded for 2.0 kg ha⁻¹ application to the main crop. This was followed by T₄ (1.5 kg ha⁻¹) and T₃ (1.0 kg ha⁻¹). The magnitude of the increase was found to be 4.2–13.5% over the control for 1.0 and 2.0 kg ha⁻¹ application to the main crop. A residual effect was not observed for the lowest level of 0.5 kg ha⁻¹ B application (Table 2). It should be mentioned that the hot water-soluble B content in the post-harvest soil of the sunflower crop was only at a sufficient level at 2.0 kg ha⁻¹. Residual effects of B on yield were reported by Dongale and Zende (1977) in wheat, while Ratna Kalyani et al. (1993) reported that the application of B to the pigeon pea crop played a critical role in reducing flower and pod drop by preventing the formation of an abscission layer. The results indicated that the soil application of 2.0 kg B ha⁻¹ not only enhanced the B uptake and yield of sunflower but also the increased the yield of green gram.

Table 2
Residual effect of sources and levels of B application on the grain and stover yields (kg ha⁻¹) of green gram

Treatments	Grain			Stover		
	S ₁	S ₂	Mean	S ₁	S ₂	Mean
T ₁	830	828	829	2293	2287	2290
T ₂	810	833	822	2307	2310	2308
T ₃	863	868	866	2433	2443	2438
T ₄	922	953	938	2547	2597	2572
T ₅	933	983	958	2627	2700	2663
T ₆	835	838	837	2190	2250	2220
T ₇	830	818	824	2133	2263	2198
Mean	860	875	868	2361	2407	2384
CD (P=0.05)	T=10.96	S=5.86	T×S=15.5	T=48.2	S=25.7	T×S=NS

NS = non-significant

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EFFECT OF SEED DRESSING AGENTS ON THE EMERGENCE OF MAIZE SOWN ON DIFFERENT DATES

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Maize seed sown in cold soil during the first ten days of April will only produce a satisfactory stand if the hybrid chosen has adequate chilling tolerance and if the seed has high biological value (germination percentage, cold test) and is treated with a high quality dressing agent.

The emergence date is influenced to a great extent by the heat sum, so only a small proportion of the considerable difference in the sowing date is manifested in the emergence date. Nevertheless, healthy plants emerging from early-sown seed have more rapid initial growth and development, as demonstrated by their greater shoot dry matter accumulation. It is not worth risking early sowing in heavily infected soil, with seed lots having poor germination ability, without adequate seed dressing, or with chilling-sensitive hybrids.

Key words: maize, seed dressing agents, emergence, different sowing dates

Introduction

The breeding and production of maize hybrids resistant to low temperature, which can thus be sown early, is necessary for reliable cultivation and the best possible exploitation of the vegetation period (Herczegh, 1978).

One important advantage of early sowing is that hybrids with longer vegetation periods and thus greater yield potential can be grown, while silking is completed before the period when water deficiency most frequently occurs, in late July and early August, leading to higher, more reliable yields and lower grain moisture at harvest (Berzsenyi et al., 1998; 1999; Marton et al., 1999; 2001). However, the greater yield potential latent in late-maturing hybrids can only be exploited safely if the genotypes have good chilling tolerance, enabling them to be sown early to make the best possible use of the long vegetation period (Sárvári and Futó, 2001).

The chilling tolerance of maize needs to be investigated up to 30–50 days after sowing (throughout the period when cold weather may occur). Chilling effects during this period may influence the germination and emergence of the seeds and the initial growth and development of the seedlings.

Breeders and geneticists generally use a combination of two temperatures – cold incubation and germination at optimum temperature – to investigate chilling tolerance, but these methods only make it possible to determine chilling tolerance in the germination stage (Marton et al., 1997).

The results of the cold test depend on many other factors, such as seed dressing, the duration of the chilling treatment, the incubation temperature and the pathogenicity of the germination medium.

The role of pathogens was demonstrated by the difference in the cold test values obtained in original maize soil and in sterilised soil (Hoppe and Middleton, 1950; McKeen, 1951; Marton, 1997b; Marton et al., 1988b). In experiments carried out by Marton (1990a) and Marton et al. (1988a) artificial inoculation with *Fusarium* species led to a reduction in the chilling tolerance of the hybrids, especially in the case of *F. culmorum* and *F. graminearum*. Hybrids were generally found to have greater tolerance to pathogens than inbred lines (Marton et al., 2000). Experiments carried out in a temperature gradient chamber also revealed that the effect of *Fusarium* infection declined at higher temperature (Marton et al., 1990).

In cold tests carried out using undressed seeds the results differed substantially depending on the pathogens present in the soil. In heavily infected soil the decisive factor determining the emergence percentages was the resistance of the genotypes to pathogens, while in less severely infected or pathogen-free soils it was the genetic chilling tolerance of the variety. For this reason the effects of chilling and pathogens must be distinguished from each other when studying genetic chilling tolerance.

In order to avoid problems caused by pathogens many authors (Kovács, 1961; Maryam and Jones, 1983; Christeller, 1984) used sterilised soil or artificial media to determine genetic chilling tolerance.

A temperature of 20–27°C is ideal for the optimum development of young maize plants. The 15–20°C temperature range is suboptimum, though the maize plants are still able to grow and develop satisfactorily. Marton (1991) found a temperature range of 9–18°C to be suitable for the evaluation and comparison of the chilling tolerance of various genotypes. Herczegh (1978) elaborated a modified cold test in which, after a 10-day cold treatment at 8°C, emergence and initial growth were tested at a temperature of 13.5°C, which is far lower than the optimum. In addition to the emergence percentage the genotypes were also characterised by the emergence rate and by a chilling tolerance index.

Herczegh and Marton (1986) investigated the germination heat minimum of hybrids and inbred lines in a temperature gradient chamber. The results of the experiments confirmed that the temperature threshold of various genotypes may differ considerably. In the suboptimum temperature range substantial differences could be demonstrated in the germination rate of the different genotypes (Marton, 1990b; 1997a; 2000a; Marton and Szundy, 1997). Stamp (1984) also observed considerable deviations in the temperature requirements of maize genotypes, which may cause unexpected difficulties in seed production due to the need to synchronise the flowering dates of parents with different vegetation periods. This draws attention to the fact that it is not sufficient to include a general temperature threshold value in maize production technologies; the minimum temperature required for emergence must be determined separately for each genotype.

In tests on the chilling tolerance of populations with different levels of heterozygosity and of their hybrids, Szundy and Marton (1999) found that the chilling tolerance of the hybrids at emergence exhibited a positive correlation with the level of heterozygosity of the female parent. In experiments carried out by Marton (2000b) it was found that the level of heterozygosity of the female parent also had an effect on the chilling tolerance of young maize plants.

Chilling tolerance tests carried out under laboratory conditions make it possible to detect differences between the genotypes, but the results obtained are not always confirmed in the field. This is due partly to the simplified climatic programmes used under laboratory conditions and partly to the fact that cold tolerance is also influenced by other environmental factors.

For this reason, in addition to phytotron experiments, a number of maize genotypes were also tested for chilling tolerance under field conditions, where the effect of soil-borne pathogens and seed dressing were also evaluated at low temperature.

Materials and methods

The experiment was set up using five maize hybrids (Norma, DK 524, Maya, Nart 150, Mv TC 287; Table 1) treated with two different dressing agents (Captan, Carboxin + TMTD and an untreated control) and sown on three types of soil (sand, maize soil infected with fusarium, heat-sterilised maize soil) at three sowing dates (April 3rd, 10th and 17th 1999). In the infected soil treatment, the effect of soil-borne pathogens was intensified by preparing a mixed suspension from 10-day-old cultures of 4 separate *Fusarium* species (*F. graminearum* Schwabe, *F. culmorum* (W.G. Smith) Sacc., *F. moniliforme* Sheldon, *F. oxysporum* Schlecht), with which the soil was sprinkled after sowing. The soil was placed in pots measuring 40 × 30 cm, with a depth of 10 cm, one soil to each pot. Thirty 10-plant plots were formed in each pot, so each pot represented two replications of each hybrid with each seed dressing treatment. Of the three sowing dates, April 17th was optimum, while sowing on April 3rd and 10th ensured a longer period of cold stress.

Prior to sowing the soil was watered to 70% water capacity. The seeds were covered with 5 cm soil, with a 1 cm sand layer on top to prevent soil cracking. To avoid water loss the pots were covered with polythene until the plants started to emerge. After this the pots were weighed and the water replaced accordingly.

The following data were recorded:

- 1) Emergence percentage: emerged plants as a percentage of the seeds sown.
- 2) Days to emergence: the emergence of individual plants was recorded daily and the weighted arithmetical mean of the number of days from sowing represented the emergence date of the plot.
- 3) Dry mass of individual shoots (mg): mass of plants dried for 48 hours at 100°C. Shoot dry mass was determined for all plants 33 days after sowing.
- 4) Shoot dry matter percentage (%).

Table 1
Properties of the maize hybrids tested in the experiments

Genotype	Type of hybrid	Vegetation period FAO	Seed germination %
Norma	SC	380	98
DK 524	SC	460	98
Maya	SC	430	97
Nart 150	TC	150	98
Mv TC 287	TC	340	97

Results and discussion

Only 4% of the undressed seeds sown in infected soil on April 3rd emerged. On heat-sterilised soil and sand the emergence percentage was 68% (Fig. 1). For dressed seeds the emergence percentage was 97% on sterilised soil, 94% on sand and 87% on infected soil. The data show that long-term cold in itself does not spoil the emergence percentage, while the pathogens destroyed almost 100% of the undressed seeds. Dressing agents had a substantial protective effect, since 87% of the seeds emerged even after artificial inoculation.

When the seeds were exposed to chilling stress for a shorter period (sowing on April 10th) the effect of the pathogens was considerably weaker: more than 80% of the undressed seeds emerged even in artificially inoculated soil. The emergence percentage of dressed seeds was around 95%. This situation did not change significantly when sowing was carried out on April 17th: dressed seeds had an emergence percentage of over 95%, while that of undressed seeds on infected soil was 81%.

The data show that a complete stand can be obtained even after sowing in early April if high quality dressing agents are used.

The number of days from sowing to emergence depended decisively on the sowing date (Fig. 2). On average, seeds sown on April 3rd emerged on the 25th day, those sown on April 10th on the 19th day and those sown on April 17th on the 13th day. In farm practice the seeds generally emerge 10–14 days after sowing.

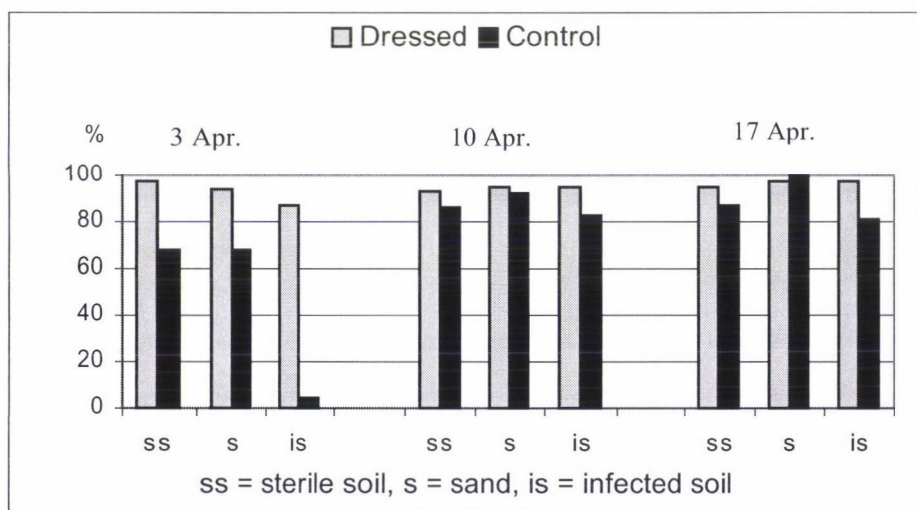


Fig. 1. Percentage emergence of maize at various sowing dates

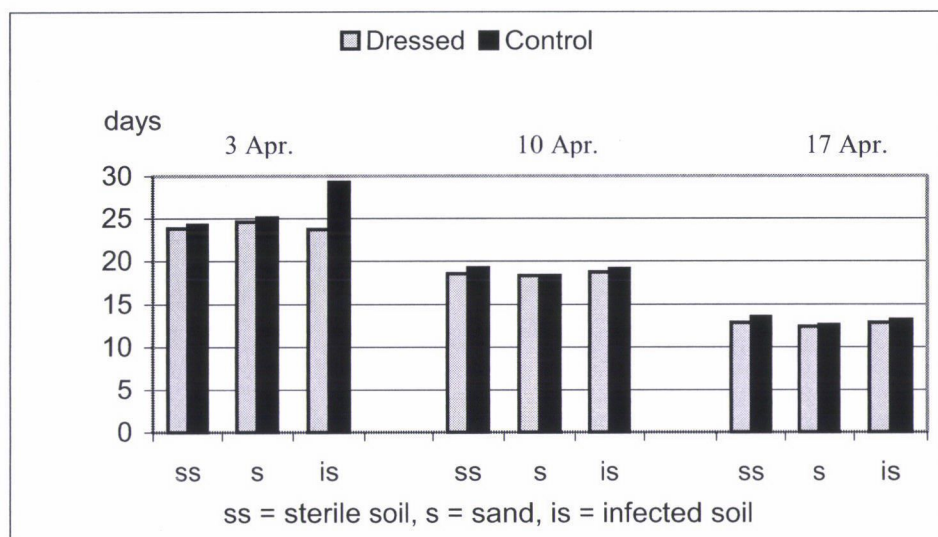


Fig. 2. Number of days from sowing to emergence after various sowing dates

When averaged over all the treatments seed dressing only shortened the time to emergence by about half a day. In the same way, the heat sterilisation of the soil also reduced the emergence time by half a day. The emergence date of undressed seeds was delayed considerably (6 days) in infected soil, especially at the first sowing date, compared to that of dressed seeds. However, this value was distorted, as it was calculated by taking the emergence time of treatments which did not germinate at all as 30 days. If the time to emergence was calculated not in days but in active heat sums (active heat sum = Σ mean $t - 10^{\circ}\text{C}$), no significant difference was found between the sowing dates: the accumulated heat sum from sowing to emergence was 45°C in the first sowing date treatment, 50°C in the second and 46°C in the third. Emergence was thus regulated principally by the temperature.

It is also worth examining which day the seeds emerged on after sowing at different dates. On average, dressed seeds sown on April 3rd emerged on April 27th, those sown on April 10th emerged on April 28–29th and those sown on April 17th on April 29–30th, so the difference was not substantial, being 1.5 days between the first and second sowing dates and 1 day between the second and third. The 14-day difference between the first and third sowing dates was reduced to 2.5 days at emergence. It should be noted that the weather was very cool in the first half of April, so that a heat sum of only 9.6°C accumulated between the first and second sowing dates (April 3–10th) and 10°C between the second and third (April 10–17th), while the 3rd sowing was followed by 5 days when the daily heat sum increment was 0. This means that for the first sowing date the active heat sum was only 19.6°C for the first 18 days, while the remainder of the total heat sum up to emergence (45°C) accumulated over the following five days.

The risk involved in early sowing (during the first 10 days of April) was illustrated by the emergence percentage of undressed seeds in infected soil, while the significant difference in the sowing date (two weeks) was not expressed in the date of emergence (2.5 days).

The effect of the sowing date was also evaluated by measuring the aboveground dry matter production of young plants from each of the sowing date treatments on the same date (May 5th).

When averaged over the seed dressing treatments the individual plant production of plants sown on April 17th was 27.1 mg, while that of plants sown a week earlier was 15% greater (31.2 mg) and that of plants sown two weeks earlier was 24% greater (33.5 mg) (Fig. 3). Although the effect of early sowing was hardly manifested in earlier emergence, the shoot dry mass accumulation was much more intensive than that of plants sown on April 17th. Greater shoot dry mass is generally associated with a stronger root system. The roots of early-sown maize penetrate to a greater depth, thus allowing the more efficient exploitation of soil moisture, leading to better protection against drought and a higher yield. The data of the non-dressed treatments, however, suggest the need for caution: the shoot dry mass of young plants from undressed seeds sown on April 17th was 20.3 mg, and that of plants sown a week earlier was considerably greater (25.8 mg), but that of plants sown two weeks earlier was the lowest of all (17.7 mg).

The dry mass of plants from undressed seeds sown in infected soil at the first sowing date was particularly low, being only 9% of that of plants from dressed seeds. Even at the second and third sowing dates plants from undressed seeds were only capable of developing 70–75% of the shoot dry mass produced by those from dressed seeds. It was interesting to note that the individual plant production of plants grown on sand was independent of the sowing date, being around 25.0 mg in dressed treatments and 20.0 mg in undressed treatments.

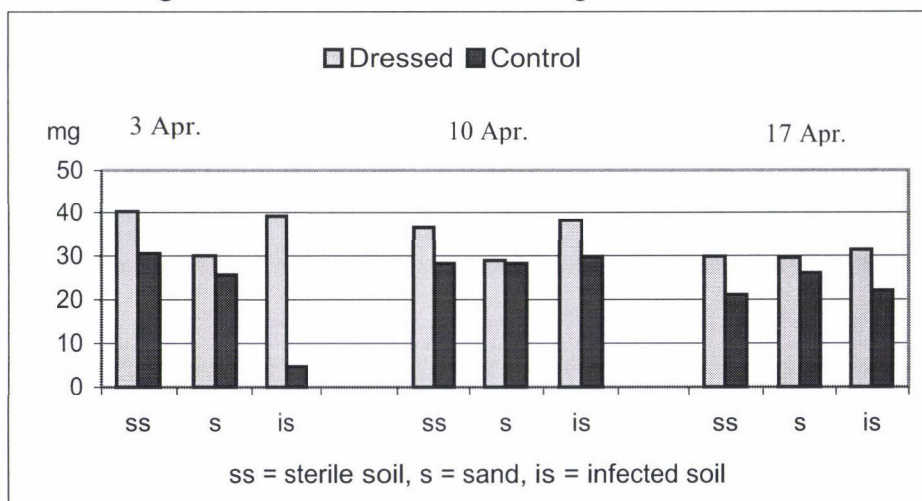


Fig. 3. Shoot dry matter production of individual maize plants after various sowing dates

It can thus be seen that soil-borne pathogens are not only capable of drastically reducing the emergence percentage, but also considerably hinder the growth and development of the emerged plants, which can thus hardly be expected to produce 100% yields.

Healthy young plants have a shoot dry matter content of around 10%, as observed in all the treatments at the third sowing date (Fig. 4). In the first and second sowing date treatments the dry matter content of plants grown on sand was higher (around 12%) than that of plants emerging in maize soil, probably due to water supply problems. Plants emerging from undressed seeds sown in infected soil on the first sowing date withered, so their dry matter content was the highest of all the treatments (14%).

The main factors having a fundamental influence on emergence characteristics were analysed in this paper. The advantages of seed dressing were perceptible for all the characters. Differences between the dressing agents are not discussed, as no difference was found for the majority of the characters (emergence time, shoot dry mass, dry matter percentage). Only for the emergence percentage was a significant difference found between the two dressing agents (Table 2). The emergence percentage of seeds treated with Carboxin + TMTD was 4.2% higher than that of those dressed with Captan. The advantage of Carboxin + TMTD was manifested in all the soils and at every sowing date.

Among the genotypes, the hybrid Nart 150 emerged earliest at all three sowing dates, while no difference was observed in emergence time between the other varieties (Table 3).

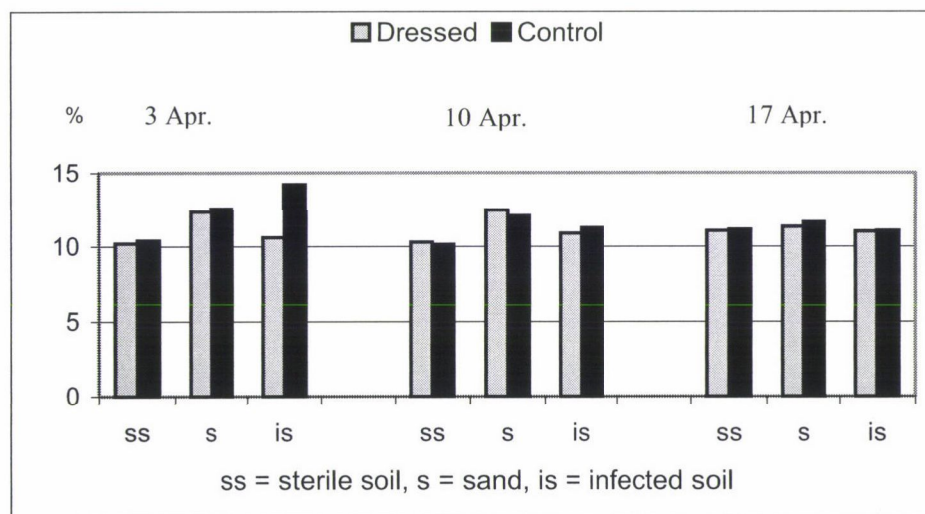


Fig. 4. Shoot dry matter content of young maize plants after various sowing dates

Table 2
Effect of seed dressing agents on the properties tested

Dressing agent	Percentage emergence	Emergence time (days)	Shoot dry mass (mg)	Shoot dry matter (%)
Captan	92.1	18.40	33.77	11.21
Carboxin + TMTD	96.3	18.42	33.64	11.08
Undressed control	74.9	19.17	23.90	11.61
LSD _{5%}	3.7	0.19	14.0	0.29

Table 3
Effect of genotype on the properties tested, averaged over sowing date, soil and dressing agent

Genotype	Emergence time (days)	Percentage emergence	Dry mass (mg/plant)
1. Norma	18.70	88.90	29.68
2. DK 524	18.76	87.60	28.25
3. Maya	18.87	88.30	33.53
4. Nart	18.20	91.70	32.27
5. Mv TC 287	18.79	82.40	28.35
LSD _{5%}	0.19	3.73	1.40

The greatest emergence percentage was also observed for Nart 150, giving a clear confirmation of the excellent chilling tolerance of this hybrid at germination and of its consequent advantage over the other genotypes. On the basis of dry mass per shoot Nart 150 was second only to Maya, indicating that Nart 150 has good chilling tolerance not only at emergence, but also in the young plant stage.

The example of Nart 150 confirms observations that earliness and chilling tolerance can be genetically combined in extra early hybrids, thus contributing to their excellent performance in cooler northern growing sites (Pintér et al., 1995a, b).

In summary it can be stated that good stands will only be obtained from maize seed sown in cold soil in the first ten days of April if the hybrid has satisfactory chilling tolerance and seeds with high biological value (germination percentage, cold test) are used after proper dressing.

The emergence time is greatly influenced by the heat sum, so only a small proportion of the difference in the sowing date is manifested in the emergence date. Nevertheless, healthy plants emerging from early-sown seed have rapid initial growth and development, which is soon exhibited in their shoot dry matter accumulation. On severely infected soil the early sowing of badly dressed seed of chilling-sensitive hybrids with poor germination ability is too big a risk to take.

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EFFECT OF SILICON AT PANICLE INITIATION ON THE GROWTH OF RICE (VAR. ADT-36) PLANTS AT DIFFERENT GROWTH STAGES

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Field experiments were conducted at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during the *Rabi* (November 1997–March 1998) and *Kharif* (July 1998–November 1998) seasons to identify the effect of silicon at panicle initiation on the growth of rice plant (Variety ADT-36) at different growth stages. Furnace slag was applied as a silicon source at 2 t/ha at the panicle initiation stage along with other nutrients. The dry matter production was recorded at the active tillering, panicle initiation, booting, flowering, one week after flowering and maturity stages in both the seasons. The total dry matter production was greater in the *Kharif* season than in the *Rabi* season. The application of slag at the panicle initiation stage along with N and K at the flowering stage had a significant influence over the dry matter production. A similar trend was observed in both the seasons. The silicon uptake was recorded at the panicle initiation and maturity stages. About 30–40% of the silicon absorbed during the early stages and the maturity stage was present in the shoot, whereas 20–30 % of the silicon absorbed during the maturity stages was present in the leaf blades. Based on the results, it is concluded that the supply of silicon during the panicle initiation stage is most important for plant growth.

Key words: rice, silicon, panicle initiation

Introduction

Although silicon has not been recognized as an essential element for higher plants, its beneficial effects have been observed in many species. Rice has been considered to be a typical silicophilous plant. It is recognized that silicon promotes photosynthesis, prevents fungal and insect injuries and alleviates lodging. Okuda and Takahashi (1965) studied the effect of silicon application on the growth of rice plants at different growth stages, and observed that the plant height, grain weight and uptake of silicon were larger when silicon was added at later growth stages (panicle initiation stage) than at early stages. They therefore suggested that silicon application is more important in the later growth stages. At the same time when the amount of nitrogen application was increased, the rice plants became more susceptible to diseases and insects and the leaves tended to droop. Increasing the amount of nitrogen application along with silicon increased the grain yield the total uptake of silicon and the total dry matter production (Shiroshita et al., 1962).

With the development of new silicon fertilizers, and with silicon materials available as waste, the additional application of silicon has become possible and

easy. It is therefore important for the economical and effective application of silicon to analyse differences in the effect of silicon on rice plants depending upon the growth stages. In this study, the growth period of the rice plant was subdivided into six stages. The effect of silicon at panicle initiation on the growth of rice plants at different growth stages was studied after the addition of furnace slag as silicon fertilizer.

Materials and methods

The cultivar Aduthurai 36 (ADT-36) was used in these experiment. The growth period of the rice plant was divided into six stages: active tillering (AT) at 20–25 days after transplanting (DAT), panicle initiation (PI, 25–35 DAT), booting (35–55 DAT), flowering (55–70 DAT), one week after flowering, and maturity.

The experiments were laid out in a randomized block design with three replications during the *Rabi* (Nov. 97–March 98) and *Kharif* (July – Nov. 98) seasons in a puddled lowland rice ecosystem. Nitrogen was applied at two levels (150 kg N ha⁻¹ and 120 kg N ha⁻¹) in four treatments each (Table 1). In all the treatments the nutrients were applied in splits at different crop stages, (basal, AT, PI and flowering stages). The recommended dose and split application of fertilizer was taken as the control plot (T1)

Samples were taken at all six stages in both the seasons. Each plant was separated into root, culm, leaf and grains. The dry weights of whole plants were determined in the six stages. Samples taken in the PI and maturity stages were used for silicon uptake estimation. The silicon content was determined by the calorimetric molybdenum blue method (Nayar et al., 1975).

Table 1
Treatment details

Treatments	Basal	AT	PI	FL
T1	N ₆₀ P ₂₅ K ₂₅	N ₂₀	N ₄₀ P ₂₅ K ₂₅	–
T2	NPK	N	NPK	N
T3	NPK	N	NP	K
T4	NPK	N	NPK, Si	–
T5	NPK	N	NK	P
T6	NPK	N	NP	NK
T7	NPK	N	NP, Si	NK
T8	NPK	N	N, Si	NPK

Si = 2 t ha⁻¹ furnace slag; AT – active tillering stage; PI – panicle initiation stage; FL – flowering stage

Results

The total dry matter accumulation was recorded at the AT, PI, booting, flowering, one week after flowering and maturity stages in both the seasons (Table 2). In general, the total dry matter production (DMP) was greater in *Kharif* than in *Rabi*.

Table 2

Total dry matter production as influenced by post-panicle nutrient management in rice (kg ha⁻¹)

Treatments	AT	PI	BOOT	F	OWAF	M
<i>Rabi 97-98</i>						
T1	1488.00	5752.87	5706.43	8973.71	8494.66	9911.53
T2	1420.97	5473.33	7596.70	10863.52	10052.39	10093.46
T3	1750.22	5063.99	6995.22	8677.22	8547.55	9462.08
T4	1704.89	5260.43	8403.49	9219.66	10364.41	11033.92
T5	1472.42	5977.31	7925.00	8908.99	8699.00	10567.83
T6	3792.89	5387.54	6594.32	9194.22	9907.44	11874.50
T7	1568.89	4845.10	8394.10	11319.67	11627.34	12547.83
T8	1765.78	5235.54	8393.65	10938.55	11399.77	11525.82
SEd	1131.02	1256.67	508.14	432.15	551.01	576.35
CD (P=0.05)	NS	NS	1089.96	927.00	1181.92	1236.28
<i>Kharif 98</i>						
T1	731.26	2831.32	5115.7	8953.19	10919.1	10962.83
T2	779.67	3585.85	5648.27	10601.99	13778.84	13962.10
T3	752.20	3369.44	5645.75	12929.07	12279.07	12594.74
T4	797.17	3994.57	6243.87	11005.58	11995.52	12524.93
T5	678.21	3748.55	5467.63	9981.98	10103.96	10266.71
T6	671.55	3548.65	6052.43	10252.11	12869.75	12954.11
T7	806.67	3470.55	6425.54	11703.74	14594.37	15108.85
T8	702.69	3424.21	5893.74	11234.42	13075.86	13287.30
SEd	47.78	245.20	384.30	625.90	763.82	572.55
CD(P=0.05)	NS	525.96	NS	1340.42	1638.10	1228.11

AT = Active tillering; PI= Panicle initiation; BOOT = Booting; F = Flowering; OWAF = One week after flowering; M = Maturity; NS = Non-significant

During *Rabi*, the treatments had a significant influence on the dry matter production (DMP) in all the stages of observation except at the AT and PI stages. At the booting stage, all the treatments registered higher DMP than the control. Maximum DMP (11319.67 kg ha⁻¹) was registered in treatment T7 at the flowering stage. Treatments T8 and T2 were at par with T7. In the one week after flowering stage, the maximum DMP (11627.34 kg ha⁻¹) was produced in T7, which was comparable with T8, T4 and T2. DMP was maximum at the maturity stage, with the highest value (12547.8 kg ha⁻¹) in the T7 treatment. This treatment was on par with T2, T4, T6 and T8. The lowest DMP (9462.08 kg ha⁻¹) was recorded in T3.

During the *Kharif* season, the treatments also had a significant impact on the dry matter production in all the stages of observations except the AT and booting stages. The T4 treatment gave the highest DMP (3994.57 kg ha⁻¹), which was at par with T2, T5 and T6 at the PI stage. During the flowering stage the maximum DMP (12929.07 kg ha⁻¹) was registered in the T3 treatment, which was on par with the T7 treatment. One week after flowering, the highest DMP (14594.37 kg ha⁻¹) was observed in T7, which was comparable with T8 and T2. Treatments T6 and T3 were also comparable (12279.07 and 12869.75 kg ha⁻¹). During the maturity stage, treatment T7 produced the highest DMP (15108.85 kg ha⁻¹), which was superior to all the other treatments. T6, T2 and

T8 produced comparable yields, while the control (T1) recorded the lowest DMP. The total dry matter production was greater in the *Kharif* season than in the *Rabi* season. The application of slag at the PI stage along with N and K at the flowering stage had a significant influence on the DM production. A similar trend was observed in both the seasons. The tiller production was highly influenced by silicon application along with the application of N and K at the flowering stage. A similar trend was observed in both the seasons. The silicon uptake was recorded at the PI and maturity stages. During *Rabi*, the culm had significantly higher silicon uptake at both the stages than the other plant parts. The highest silicon uptake ($227.63 \text{ kg ha}^{-1}$) was observed in T7, which involved the application of N and K at flowering and slag at the PI stage, and this was comparable with the T8 treatment ($226.15 \text{ kg ha}^{-1}$) in both the seasons. Treatments to which slag was not applied showed lower silicon uptake at the maturity stage (Table 3). During both the seasons, the root and leaves were not influenced by the application of slag at the PI stage. At the maturity stage T7 exhibited high silicon uptake (87.84 kg ha^{-1}) which was on par with T8 and T4. The other treatments registered lower silicon uptake and were comparable.

During the *Kharif* season, the highest silicon uptake was recorded in T7 ($229.06 \text{ kg ha}^{-1}$) in the culm at the maturity stage. The treatments had a similar effect as in the *Rabi* season in influencing the silicon uptake.

Table 3
Silicon uptake as influenced by post-panicle initiation nutrient management in rice (kg ha^{-1})

Treatments	PI			M		
	Root	Culm	Leaf	Grain	Culm	Leaf
<i>Rabi 97-87</i>						
T1	1.23	10.35	6.31	36.40	65.01	13.35
T2	2.08	12.37	6.13	54.32	104.00	16.42
T3	1.91	16.20	8.24	55.22	103.71	15.52
T4	1.58	17.66	14.08	80.41	171.67	26.93
T5	2.43	17.21	6.00	45.02	94.21	15.85
T6	1.85	14.09	7.28	49.60	97.22	12.91
T7	1.77	19.85	4.67	87.84	227.63	31.45
T8	2.06	17.5	5.57	85.04	226.15	31.94
SEd	0.43	1.09	1.81	8.21	28.30	2.56
CD (P=0.05)	NS	2.34	NS	17.61	60.70	5.48
<i>Kharif 98</i>						
T1	1.35	10.57	6.47	37.78	66.10	14.39
T2	2.18	12.80	6.29	55.69	105.06	17.42
T3	2.00	17.20	8.39	55.93	106.10	16.52
T4	1.65	18.80	11.37	81.09	173.05	28.28
T5	2.48	17.45	6.69	45.40	95.32	16.55
T6	1.98	14.33	8.31	50.95	98.26	13.69
T7	1.90	20.00	5.37	88.87	229.06	32.86
T8	2.15	17.75	6.35	86.06	226.94	33.31
SEd	0.43	1.21	1.88	8.31	28.05	2.61
CD(P=0.05)	NS	2.59	NS	17.83	60.16	5.60

PI= Panicle initiation; M = Maturity; NS = Non-significant

Discussion

The production of biomass continued till maturity due to continued photosynthesis. The partitioning into the leaf, culm and root was not seen clearly after flowering during the *Rabi* season, but during the *Kharif* season, the culm and root production continued up to the one week after flowering stage. In both the seasons, the culm growth was reduced during the maturity stages. The production of dry matter continued after panicle emergence mainly by the remobilization of nutrients in the soil due to continued photosynthetic activity.

The rhizosphere of rice plants becomes reductive from about panicle initiation to the heading stage (Alberda, 1954). At this time the demand of rice plants for N is higher. With the existing practice of applying N at the basal, AT, and PI stages the plants are subjected to decreased availability owing to various losses and absorption by the rice plants. This might develop a gap between the N requirement of the rice plant at and after flowering and the availability in the soil. The lower uptake of rice plants estimated from the existing practices of nutrient management could be related to the lower availability of N at later stages. Therefore, the application of N after panicle initiation had an impact on the dry matter production at later stages. In rice soils P fixation occurs frequently, and it is required more during the PI to flowering stages. Hence the application of P at the basal and flowering stages had a great impact on the dry matter production. This crop requires large quantities of potassium. A sustained supply up to flowering had a great influence on the plant dry matter production (Yoshida, 1981).

The application of slag with other nutrients at the flowering stage had a clear influence on the root production and N uptake. The root N uptake estimated at around PI showed a low value, probably due to the reduced soil conditions. As reported by De Datta (1981), it could be assumed that in a clayey soil, the soil redox conditions decline sharply at around 5–6 weeks after submergence. The application of slag and the irrigation method followed, involving irrigating one day after the disappearance of ponded water, at the beginning of PI, might have alleviated the soil conditions to some extent, if not completely. The beneficial effect of slag on the plants was seen during the experimental periods. At the PI stage, the culm showed a higher silicon content than the leaf and grain, but at the maturity stage grain received a considerable amount of silicon from the other vegetative parts. This was particularly true on the slag-treated plots. There was also an increase in the uptake of Si over the control due to the addition of phosphorus at the PI stage. Similar results were reported by Lee (1983).

Based on the experimental results it is concluded that the application of silicon along with N and K at the flowering stage resulted in higher dry matter production and increased silicon content in the plants.

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Short communication

MODIFIED JOINT SCALING TEST FOR EVALUATING THE EFFECT OF THE LEVEL OF HETEROZYGOSITY OF THE FEMALE PARENT

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In earlier studies the inheritance of chilling tolerance in maize was investigated using the joint scaling test on six genotypes forming a systematic genetic series – P_1 , P_2 , F_1 , F_2 , B_1 , B_2 . The values of some genotypes (P_1 , P_2 , F_1) were overestimated by the model, while those of the other genotypes (F_2 , B_1 , B_2) were underestimated. It was thought that this could be due to the effect of the level of heterozygosity in the female parent. The level of heterozygosity of the female parent in the P_1 , P_2 , F_1 genotypes is 0%, while in the F_2 , B_1 , B_2 genotypes it is 100%. In addition to the m , $[d]$ and $[h]$ parameters, a new parameter, $[fh]$ (female heterozygosity) was thus introduced. Analysis carried out with the new model confirmed a significant female heterozygosity effect.

Key words: joint scaling test, cold tolerance, additive-dominance effect, maize

Introduction

The scaling test described by Cavalli (1952) and Mather and Jinks (1974) has been successfully applied by many authors to study the inheritance of various characters in maize. When evaluating the inheritance of chilling tolerance Maryam and Jones (1983) found that the model could be applied to the data of many genetic series. Marton (1990) also observed that the additive-dominance model was suitable for the investigation of maize chilling tolerance, but it was found that the model systematically overestimated the value of some genotypes, while underestimating that of others. The values measured for P_1 , P_2 and F_1 were generally lower than those estimated with the model, while those measured for F_2 , B_1 and B_2 were higher.

A reference to the role of female heterozygosity in chilling tolerance can be found in the work of Szundy and Kovács (1981a, b), who found that three-way cross hybrids developed from female parents with a 100% level of heterozygosity emerged more rapidly and at a higher percentage than single cross hybrids developed on female parents with a 0% level of heterozygosity.

The present paper discusses how the level of heterozygosity of the female parent, as a parameter involved in the genetic determination of maize chilling tolerance, can be included in the joint scaling test.

Materials and methods

The difference between the original and modified joint scaling tests is demonstrated on the data of Marton (1990) and Mather and Jinks (1974). The former refer to the leaf area (cm^2/plant) of six genotypes of a systematic genetic series developed using two lines (CM 174, Mo 17) with different levels of chilling tolerance (Herczegh and Marton, 1986, Marton et al., 1997). Seedlings of these genotypes were grown in a phytotron chamber at 12°C for 26 days, after which the leaf area of the plants was measured using a Hayashi Denco instrument.

Results and discussion

When these data were evaluated with the original model (Table 1) the results of the Chi^2 test indicated that the joint scaling test could not be applied, since the values measured for the genotypes and those estimated with the model differed considerably from each other (Table 1). As in earlier investigations, the real values of P_1 , P_2 and F_1 were lower than the estimated data, while those of F_2 , B_1 and B_2 were higher.

This grouping of the genotypes was thought to be due to a factor not included in the model, namely the level of heterozygosity of the female parent, which is 0 % in the case of P_1 , P_2 and F_1 and 100 % for F_2 , B_1 and B_2 .

In order to determine whether the level of heterozygosity of the female parent really influenced the chilling tolerance of the young plants the joint scaling test was modified as follows.

A new parameter, $[\text{fh}]$ (female heterozygosity) was introduced, which can be determined in the same way as $[\text{h}]$. In the present case the female parents of the P_1 , P_2 and F_1 genotypes were inbred lines, with a heterozygosity level of 0%, ($[\text{fh}] = 0$). The female parents of the F_2 , B_1 and B_2 genotypes were F_1 hybrids, with a heterozygosity level of 100%, giving an $[\text{fh}]$ value of 1 (Table 2). Both the m , $[\text{d}]$ and $[\text{h}]$ values and the analysis itself satisfy the criteria laid down by Cavalli (1952) and Mather and Jinks (1974). First the genetic parameters were

Table 1
Evaluation of the inheritance of maize leaf area using the joint scaling test

Genotype	Measured	Estimated	Difference
P_1	6.410 ± 0.858	7.030	-0.620
B_1	22.900 ± 1.765	17.871	5.029
F_1	21.050 ± 2.581	28.712	-7.662
F_2	22.100 ± 3.515	20.373	1.727
B_2	27.000 ± 2.748	22.876	4.124
P_2	15.790 ± 2.015	17.041	-1.251
Genetic parameters			
m	12.035 ± 1.043		
$[\text{d}]$	-5.005 ± 1.032		
$[\text{h}]$	16.676 ± 2.357		
Chi^2	20.332 ^{xxx}		
P	1.40%		

estimated, after which these were used to estimate the genotype means, which were then compared with the actual data. The applicability of the model was then tested on the basis of the sum of squares of the estimated and measured data using the χ^2 test, in the present case with $FG=2$ degrees of freedom (No. of generations – No. of parameters).

Six equations were compiled for the estimation of the m , $[d]$, $[h]$ and $[fh]$ parameters on the basis of Table 2. As there were more equations than unknowns, the estimation was carried out using the least squares technique, as described by Mather and Jinks (1974). Since the means of the various generations were not known with equal precision, the generation means were weighted with the reciprocals of the squared standard errors.

The six equations and their weights can be combined in the following manner to give four equations for the estimation of the weighted least squares of the four parameters. Each of the six equations is multiplied by the coefficient of the parameters they contain (Table 2) and by their weights (reciprocal of the standard error squares). The values of the parameters (columns) and genotypes (rows) are then summed to give four equations which are solved by means of matrix inversion. The equations thus give the values of the four parameters and their variance, the square root of which is the standard error. Using the standard error or the t -test it is possible to check whether the parameters differ from zero or not.

The χ^2 test on the analysis carried out with this model illustrates the applicability of the model (Table 3). The parameters tested showed a highly significant difference from zero. The effect of the $[fh]$ parameter was thus statistically confirmed. The estimated and measured data were very close to each other. The differences were substantially smaller than those given by the original model.

In order to check whether the model erred in the case of characters where the female parent or the level of heterozygosity of the female parent is unlikely to have any effect of the development of the character, such as the number of loculi/fruit in tomatoes, the analysis was run on the 1938 data of the Johannisfeuer \times Danmark cross (Powers, 1941) used as an example by Mather and Jinks (1974).

Table 2
Genetic parameters of genotypes analysed using the modified joint scaling test

Genotype	m	$[d]$	$[h]$	$[fh]$
P_1	1	1	0	0
B_1	1	1/2	1/2	1
F_1	1	0	1	0
F_2	1	0	1/2	1
B_2	1	-1/2	1/2	1
P_2	1	-1	0	0

Table 3
Evaluation of the inheritance of maize leaf area using the modified joint scaling test

Genotype	Measured	Estimated	Difference
P ₁	6.410±0.858	6.442	-0.032
B ₁	22.900±1.765	22.311	0.589
F ₁	21.050±2.581	21.050	0.000
F ₂	22.100±3.515	24.604	-2.504
B ₂	27.000±2.748	26.896	0.104
P ₂	15.790±2.015	15.612	0.178
Genetic parameters			
m	11.027±1.067		
[d]	-4.585±1.037		
[h]	10.023±2.793		
[fh]	8.565±1.930		
Chi ²	0.629		
P	73.0%		

The analysis (Table 4) shows that the model is applicable, and that the Chi² value (0.920) is even lower than that obtained with the original model (4.370) (Table 5), although the value of the [fh] parameter (0.129±0.070) was negligible compared with that of [d] (1.844) or [h] (-1.808). On the other hand, the values of the genetic parameters estimated by the original model (m: 7.341, [d]: 1.847, [h]: -1.742) (Table 5) did not change to any great extent when the modified model was applied (m: 7.308, [d]: 1.844, [h]: -1.808). It can thus be seen that the modified model only exhibited an [fh] effect when such an effect really existed.

In summary it can be concluded that the expansion of the joint scaling test to include the [fh] parameter makes it suitable for demonstrating the effect of the level of heterozygosity of the female parent, which can be expected to be felt mainly in characters scored at emergence or in the seedling stage.

Table 4
Evaluation of the inheritance of the number of loculi per fruit in tomato using the modified joint scaling test (Johannisfeuer × Denmark 1938 data from Powers 1941)

Genotype	Measured	Estimated	Difference
P ₁	9.125±0.091	9.153	-0.028
B ₁	7.500±0.100	7.456	0.044
F ₁	5.500±0.086	5.500	0.000
F ₂	6.595±0.118	6.533	0.062
B ₂	5.575±0.064	5.600	-0.036
P ₂	5.475±0.057	5.464	0.011
Genetic parameters			
m	7.308±0.053		
[d]	1.844±0.049		
[h]	-1.808±0.101		
[fh]	0.129±0.070		
Chi ²	0.920		
P	63.1%		

Table 5

Evaluation of the inheritance of the number of loculi per fruit in tomato using the original joint scaling test (Data from Mather and Jinks 1974)
(Johannisfeuer \times Danmark 1938 data from Powers 1941)

Genotype	Measured	Estimated	Difference
P ₁	9.125 \pm 0.091	9.188	-0.063
B ₁	7.500 \pm 0.100	7.393	0.107
F ₁	5.500 \pm 0.086	5.599	-0.099
F ₂	6.595 \pm 0.118	6.470	0.125
B ₂	5.575 \pm 0.064	5.546	-0.029
P ₂	5.475 \pm 0.057	5.494	-0.019
Genetic parameters			
m	7.341 \pm 0.050		
[d]	1.847 \pm 0.049		
[h]	-1.742 \pm 0.094		
Chi ²	4.370		
P	30-20%		

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Short communication

HIGH *IN VITRO* SHOOT PROLIFERATION IN THE APPLE CULTIVAR JONAGOLD INDUCED BY BENZYLADENINE ANALOGUES

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The *in vitro* shoot multiplication of apple cv. Jonagold was tested on media containing benzyladenine, benzyladenine riboside or meta-topolin in different concentrations (from 0.0 to 5.0 mg l⁻¹). The optimal concentration for the best multiplication varied according to the type of cytokinin. The highest multiplication rate (on average 6.9 and 5.9 new shoots per explant) was achieved using 5.0 mg l⁻¹ meta-topolin or 2.0 mg l⁻¹ benzyladenine riboside. The longest shoots were formed on media containing benzyladenine riboside at a concentration of 0.5 mg l⁻¹. The length of newly developed shoots was strongly suppressed by high concentrations of different cytokinins, but the suppression effect of a high concentration of meta-topolin on shoot length was less than that of benzyladenine or benzyladenine riboside. In this study meta-topolin and benzyladenine riboside proved to be effective cytokinins to induce adequate shoot proliferation, while benzyladenine was the least active cytokinin.

Key words: apple, meta-topolin, benzyladenine, benzyladenine riboside, shoot multiplication

Introduction

Although benzyladenine (BA), one of the most effective cytokinins, is generally used as cytokinin in apple micropropagation systems (Jones et al. 1977; Lane, 1978; Sriskandarajah and Mullins, 1981) it might have disadvantages such as difficulties in rooting (Webster and Jones, 1991) or toxicity after several subcultures on the same medium (Werner and Boe, 1980). The side effects of benzyladenine may be due to N⁷- and N⁹-glycosilation or alanin conjugation resulting in biologically inactive but chemically very stable derivatives. The slow release of benzyladenine from its derivatives might cause the inhibition of rooting as proved in other plant species (Werbrouck et al., 1995).

The use of benzyladenine derivatives which are already conjugated at the N⁹-position, such as benzyladenine-9-riboside (BAR), or hydroxylated benzyladenine analogues, such as meta-topolin (N⁶-meta-hydroxy-benzyladenin, TOP) could be an alternative way to avoid the side effects of benzyladenine. Hydroxylated BA analogues have a metabolism distinct from that of BA. Meta-topolin is a newly isolated and highly active natural growth substance (Strnad et al., 1997).

The aim of this study was to test the response of cv. Jonagold to different cytokinins in order to find out whether benzyladenine-9-riboside and metatopolin could be used effectively for shoot multiplication.

Materials and methods

In vitro cultures of cv. Jonagold scions were established as reported earlier (Dobránszki et al., 2000a). Shoots were multiplied on MS medium (Murashige and Skoog, 1962) containing 100 mg l⁻¹ myo-inositol, 0.7% agar-agar and 3% saccharose, supplemented with 1.0 mg l⁻¹ benzyladenine riboside, 0.3 mg l⁻¹ indole-3-butyric acid (IBA) and 0.2 mg l⁻¹ gibberellic acid (GA₃) before the shoot multiplication experiments. The response of cv. Jonagold to different concentrations (0.0, 0.5, 2.0, 3.5 and 5.0 mg l⁻¹) of BAR, BA and TOP was then tested at constant IBA level (0.1 mg l⁻¹). Other components of the medium were the same as for shoot multiplication. The pH of the medium was adjusted to 5.8 before autoclaving for 20 min at 121°C and 10⁵ Pa. Four shoot cuttings (40.0 mm) were placed on 40 ml of the various media in Kilner jars and the cultures were grown at 22±2°C with a 16 h photoperiod provided by warm-white lamps (Tungsram) at a PPFD of 105 µmol s⁻¹m⁻². Each treatment consisted of at least 40 explants and the experiments were repeated twice. The number and length of newly developed shoots were observed after four weeks. The statistical analysis involved analysis of variance followed by Tukey's test, using the SPSS 7.5 for Windows program.

Results and discussion

Data on the multiplication rate are presented in Table 1. The highest proliferation rates were obtained on media supplemented with 5.0 mg l⁻¹ metatopolin or 2.0 mg l⁻¹ BAR, which produced 6.9 and 5.9 shoots per explant, respectively. Explants cultured on media without cytokinin tended to form only one new shoot (on average 1.6 shoots per explant) confirming that cytokinins are necessary for adequate shoot proliferation (Lane, 1978; Sriskandarajah et al., 1990; Baraldi et al., 1991).

The optimal concentrations for shoot multiplication varied for the different cytokinins. In general, the proliferation rate of *in vitro* apple shoots was reported to be markedly influenced by the concentration of BA (James and Thurbon, 1981; Lane and Looney, 1982; Lane and McDougald, 1982; Welander, 1985). Even though a relatively wide range of BA concentrations was used in the present experiments, the number of newly developed shoots was not affected by the BA levels for cv. Jonagold (it varied from 3.5 to 4.1 new shoots per explant). The cv. Jonagold had already seemed to be insensitive to different BA levels in previous experiments where 0.5 and 1.0 mg l⁻¹ were tested, resulting in 5.6 and 5.2 new shoots per explant, respectively (Dobránszki et al., 2000b).

When BAR was used as cytokinin, the 2.0 mg l⁻¹ concentration proved to be optimal as regards the number of new shoots. The results of previous experiments with cv. Jonagold, where 0.5 and 1.0 mg l⁻¹ BAR were tested and gave 3.2 and 6.5 new shoots per explant, suggested that BAR could be a very effective cytokinin but only at the higher concentration (Dobránszki et al.,

2000b). The present study showed a peak at 2.0 mg l^{-1} , suggesting that a further increase in BAR concentration up to 2.0 mg l^{-1} could result in a higher multiplication rate. However, the results of proliferation rates in the most recent experiments were lower than in previous experiments, irrespective of whether BA or BAR was used as cytokinin. One reason for the differences in the number of new shoots could be that the auxin levels were different: in the present study the media contained 0.1 mg l^{-1} instead of 0.3 mg l^{-1} IBA and the auxin level could modify the multiplication rate depending on the genotype (Dobránszki et al., 2000a).

The highest frequency of new shoot formation was observed on medium containing 5.0 mg l^{-1} meta-topolin. The multiplication rate increased as the meta-topolin concentration was increased in the medium. Werbrouck et al. (1996) also found meta-topolin to be very effective for the shoot multiplication of *Spathiphyllum floribundum*, but only at a relatively high concentration.

When different cytokinins were compared at the same levels BA and BAR were found to be the most effective at the lowest concentration (0.5 mg l^{-1}). However, at higher concentrations (2.0 and 3.5 mg l^{-1}) BAR and TOP resulted in the best multiplication rates, while only TOP proved to be the most effective at the highest level (5.0 mg l^{-1}). In these experiments BA was the least active cytokinin.

The newly developed shoots were relatively short in all the treatments (Table 2). Although the longest shoots (33.8) were produced on medium with 0.5 mg l^{-1} BAR, the length of shoots grown on media with 0.5 mg l^{-1} TOP and BA did not differ from this significantly. Thus the longest shoots were observed on media containing cytokinins in the lowest concentration, independently of the type of cytokinin. As the cytokinin concentration increased, the length of the shoots decreased in each treatment. If the concentration of BA and BAR was higher than 3.5 mg l^{-1} , shorter shoots were produced than on medium without cytokinin. When comparing different concentrations of BA, Welander (1985) also found that longer shoots with larger leaves developed on media with lower levels of BA. Similarly, Marin et al. (1993) reported the shortening and thickening of shoots at high concentrations of BA.

Table 1

Effect of different types and concentrations of cytokinin on the number of new shoots per explant (=multiplication rate)

Type of cytokinin	Concentration of cytokinin (mg l^{-1})				
	0.0	0.5	2.0	3.5	5.0
Number of new shoots per explant					
Benzyladenine	1.6a	4.1b B	3.5b A	3.5b A	3.9b A
Benzyladenine riboside	1.6a	4.3b B	5.9c B	5.6c B	4.6b A
Meta-topolin	1.6a	3.4b A	5.3c B	5.6c B	6.9d B

Different small letters in each row and capital letters in each column indicate significant differences ($P < 0.05$) between the treatments.

Table 2
Effect of types and concentrations of cytokinin on the length of shoots

Type of cytokinin	Concentration of cytokinin (mg l ⁻¹)				
	0.0	0.5	2.0	3.5	5.0
Length of new shoots (mm)					
Benzyladenine	15.5ab	30.6c A	18.1b A	16.3ab A	14.8a B
Benzyladenine riboside	15.5b	33.8d A	21.9c B	17.1b A	12.0a A
Meta-topolin	15.5a	31.4d A	25.1c C	22.7bc B	20.9b C

Different small letters in each row and capital letters in each column indicate significant differences ($P < 0.05$) between the treatments.

Differences could be observed in the suppression of shoot length in cv. Jonagold by high levels of different types of cytokinins. At the highest concentration the shoot lengths were 35.5, 48.4 and 66.6% of the maximum in the case of BAR, BA and TOP, respectively. Werbrouck et al. (1996) also found the highest frequency of long shoot formation on media with TOP compared to BA in multiplication experiments with *Spathiphyllum floribundum*.

It can be concluded that shoot multiplication could be increased by using BAR, a BA derivative conjugated at the N⁹-position by ribose, but its suppression effect on shoot length increased markedly with an increase in concentration. However, the shoot length of new shoots was satisfactory when BAR was used at the 2.0 mg l⁻¹ concentration, which was optimal for the multiplication rate.

These results suggest that meta-topolin could be a very effective cytokinin in apple micropropagation because it could significantly increase the multiplication rate with the least suppression effect on shoot length. Further research is necessary to find the optimal concentration and to test the responses of other cultivars.

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Short communication

LENTIL (*LENS CULINARIS* MEDIC.) PRODUCTIVITY AS INFLUENCED BY RATE AND METHOD OF PHOSPHATE PLACEMENT IN A MEDITERRANEAN ENVIRONMENT

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The influence of rates and methods of phosphate placement on the productivity of lentil (*Lens culinaris* Medic.) was studied in a two-field experiment. Each experiment consisted of four levels of phosphorus (0, 17.5, 35.0 and 52.5 kg P ha⁻¹) and two methods of placement (banding and broadcast). Lentils grown at Kufran had higher seed yield than those grown at Houfa. Seed yield, pods plant⁻¹ and branches plant⁻¹ increased significantly in both locations after phosphorus application compared with the control. Seed yield was significantly greater with band placement than with the broadcast method of phosphorus application at both locations.

Key words: *Lens culinaris* Medic., phosphorus rate, broadcast, band, seed yield

Introduction

Lentils are an important protein source for the people in Jordan and in countries of West and South Asia and North Africa. The crop is grown under rainfed conditions and in rotation with cereals such as wheat and barley. Lentils are usually grown as a winter crop, on marginal lands where rainfall is low and soil productivity is poor.

The area planted to lentils in Jordan has decreased by more than 50% in the last 10 years (1990–1999) and the yield has also significantly decreased. At the same time imports are growing fast. The imports of lentil seeds increased to 5,000 tons in 1999 with a value of 2.5 million US\$ (FAO, 1999).

Leguminous crops require an adequate supply of readily available nutrients for optimum growth and yield. Legume crops can be quite responsive to P fertilization, particularly where soils test low in available P.

Soil analyses have shown that phosphate deficiency is widespread in the calcareous soils which comprise over half of the total cultivated area of the Mediterranean region (Kassam, 1981). Field trials conducted on these soils have demonstrated large and economical response to phosphate fertilizers (Cooper, 1983; Harmsen, 1984).

The method, quantity and time of phosphate application should be carefully chosen to obtain profitable results in crop growth. In Australia, phosphate is applied to the surface of the soil, although there have been few studies on the relative effectiveness of this method for crop and pasture species

(Ozanne et al., 1976). The advantage of drilling superphosphate compared with broadcasting has been reported by Loutit et al. (1968) for cereal crops. However, the information on legume crops is very limited. Currently, most farmers who use phosphate on legume crops broadcast and incorporate the fertilizer prior to seeding. However, several authors have demonstrated that the method of placement could affect the phosphate recovery and yield obtained (Barber, 1979; Cope, 1981; Randall and Hoeft, 1988; Sander et al., 1990; Webb et al., 1992).

The effect of the rate and method of P application on the plant growth of cereal crops has been investigated for many soils and environments but information pertaining to calcareous soils in the Mediterranean region for legume forage crops is still very scarce, despite the fact that these soils occupy a high proportion of the cultivated areas. The objective of the present investigation was to study the effect of the rates and methods of phosphate placement on the seed yield of lentils and on the recovery of phosphate fertilizers under different soil and environmental conditions in Jordan.

Materials and methods

Two fields at Houfa and Kufran in north Jordan were used in this study. The soil where the plants grew in Houfa was rocky, shallow and silty (fine, mixed, thermic, typic xerochrept with a pH of 8.1 and available phosphorus ranging from 23 to 60 kg ha⁻¹). The soil at Kufran was silty clay with a pH of 7.8 and available phosphorus ranging from 22 to 55 kg ha⁻¹. Each experiment consisted of three replicates of a factorial design with four levels of phosphorus (0, 17.5, 35.0 and 52.5 kg P ha⁻¹) and two methods of placement (banding and broadcast). Phosphorus was supplied in the form of triple superphosphate (21% P). The phosphate fertilizer was either drilled with the seed after cultivation (banded) or broadcast and rotovated into the surface layer just before sowing (broadcast). Lentil seeds were sown in rows 22.5 cm apart at a rate of 80 kg ha⁻¹. The plot size was 1.8 × 2 m. Nitrogen fertilizer was applied uniformly by hand across all treatments [20 kg N ha⁻¹ at sowing in the form of urea (46% N) and 40 kg N ha⁻¹ top-dressed at the start of flowering]. Weeds were controlled by hand as needed. At maturity, 1 m² from each plot was cut about 5 cm from the soil surface to be dried and weighed. After threshing, seed and straw weights were recorded separately.

The measured variables were seed yield (kg ha⁻¹), 100 seed weight (g), seeds pod⁻¹, branches plant⁻¹, plant height (cm) and days to heading (day). The analysis of variance and LSD mean separation were performed using the MSTAT-C statistical computer program as described for a split-plot design by Steel and Torrie (1980). Comparisons between means were made using the least significant difference test (LSD) at the 0.05 probability level.

Results and discussion

Lentils (*Lens culinaris* Medic.) responded well to phosphorus application and placement methods. The interaction effects for the levels of phosphorus and the placement methods, however, were not significant in respect of different variables at either location.

Lentils grown at Kufran had higher seed yield than those grown at Houfa (Table 1). The higher seed yield may have been due to the favourable moisture and soil depth as well as to the longer life cycle of the plants at Kufran compared with Houfa. In addition, the deep soil at Kufran might have retained moisture for longer periods than the shallow soils at Houfa to lengthen the plant life cycle at

the Kufran location compared to Houfa. Moreover, the plants at Houfa flowered and reached physiological maturity earlier than those at Kufran. Many breeding strategies for yield increases are based on the assumption that increased yield potential depends on increases in the size of the photosynthetic source, achieved through lengthening the growth cycle (White et al., 1991). Increases in crop yield arise from the amount of solar radiation plants intercept to lengthen the life of the canopy. Lengthening the life of plant canopies has been associated with increased yields (Gifford and Evans, 1981).

Phosphorus rates

At low rate of applied phosphorus (P0 and P1) lentils exhibited phosphorus deficiency symptoms, including dwarf growth and purpling of the leaves. Such effects were absent at high phosphorus rates (P3 and P4). Similar results were found by Turk (1997). In both locations, an increase in P application generally increased the seed yield and branches plant⁻¹, and reduced the 100-seed weight. Seeds pod⁻¹ was higher at P2 and P3 than at either P0 or P1. However, the differences between P2 and P3 were not significant for seed pod⁻¹ at either location and not significant for plant height at Houfa. The effect of P significantly increased seed yield due to the fact that P increased the consumptive use of moisture and moisture use efficiency (MUE). This was attributed to more root extension and more soil moisture utilization. Similar results were reported by Ali-Khan and Kiehn (1989), who found that P application to lentils increased seed yield. Days to 50% flowering decreased significantly in both locations after phosphorus application compared with the control (Table 2). This could be attributed to the fact that phosphorus fertilizer application increased the rate of crop development from emergence to floral initiation and advanced anthesis. Similar results were reported by Keatinge et al. (1985) who found that phosphorus fertilizer decreased the number of days to 50 % flowering.

Table 1

Seed yield (kg ha⁻¹), 100 seed weight (g) and seeds pod⁻¹ as influenced by rates and methods of P application.

Treatments	Seed yield (kg ha ⁻¹)		100-seed weight (g)		Seeds pod ⁻¹	
Methods	Houfa	Kufran	Houfa	Kufran	Houfa	Kufran
Broadcast	782.5	835.0	57.0	57.3	1.4	1.7
Band	845.0	927.5	56.8	56.5	1.4	1.7
LSD	61.0	77.0	NS	NS	NS	NS
Rates						
P0	675.0	725.0	60.0	60.5	1.0	1.0
P1	780.0	840.0	58.0	58.5	1.3	1.7
P2	835.0	925.0	56.0	55.5	1.7	2.0
P3	965.0	1035.0	53.5	53.5	1.7	2.0
LSD	48	45	1.8	1.9	0.3	0.4
Interaction	NS	NS	NS	NS	NS	NS

NS: not significantly different according to LSD ($P \leq 0.05$).

Table 2

Branches plant⁻¹, plant height (cm), and days to heading (day) as influenced by rates and methods of P application

Treatments	Branches plant ⁻¹		Plant height (cm)		Days to heading (day)	
Methods	Houfa	Kufran	Houfa	Kufran	Houfa	Kufran
Broadcast	3.0	4.1	34.3	37.5	91.5	98.0
Band	3.6	4.3	36.7	40.4	91.3	98.0
LSD	0.5	0.2	2.1	2.7	NS	NS
Rates						
P0	2.3	3.5	32.2	35.6	98.0	103.0
P1	2.9	4.0	35.0	37.8	93.0	100.0
P2	3.9	4.3	37.3	40.2	89.5	97.0
P3	4.2	5.0	37.5	42.3	85.0	92.0
LSD	0.3	0.4	1.7	2.2	3.0	2.7
Interaction	NS	NS	NS	NS	NS	NS

NS: not significantly different according to LSD ($P \leq 0.05$).

Placement methods

Seed yield and plant height were significantly greater with band placement than with the broadcast method of phosphorus application at both locations (Table 1). Branches plant⁻¹ was significantly greater with band placement than in the broadcast method at the Kufran location. Seed pod⁻¹ and heading date, however, did not exhibit any marked variation in either location.

The superiority of band placement was probably due to a combination of higher available soil moisture and a greater probability of roots being exposed to the fertilizer.

It is thus inferred from the above results that the seed and dry matter of lentils (*Lens culinaris* Medic.) can be increased further with the addition of P to soils with medium available P status (10 mg Olsen's P kg⁻¹ soil). The results also showed a significant effect of the P placement method.

In general, P application by band placement is more effective because it was more likely to be in contact with moist soil. Moreover, band placement increases the probability of root contact, as roots tend to grow downward in legumes.

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Short communication

GENOTYPE \times ENVIRONMENT INTERACTION OF SEED YIELD AND SEED INDEX IN BITTER VETCH

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Nine bitter vetch selection lines were evaluated in three successive years to determine their yield and seed index (100-seed weight) stabilities, based on three parameters: phenotypic index (P), regression coefficient (bi), and least deviation from regression (S^2 di). The line Sel. 2517 (L7) was identified as the most stable one for the growing seasons, while Sel. 2509 (L2) and Sel. 2511 (L4) were found to be stable for seed yield under favourable climatic conditions. For seed index Sel. 2515 (L6) was identified as the most stable line. Selection line 2513 (L5), which originated from Cyprus, had the highest degree of responsiveness to changing environments.

Key words: bitter vetch, yield, seed index, genotype \times environment interaction

Introduction

Vetches (*Vicia* species) are common forage legumes in the rainfed, semi-arid systems of the Mediterranean region. They are used for high quality hay production, for grazing after the beginning of the pod formation stage, or for seed and straw production, and may be grown in a monoculture (Droushiotis, 1985) or in mixtures with cereals (Osman and Nersoyan, 1986). Both low rainfall and low temperature limit the growth of vetch in the Mediterranean region and, as a result, the final production is lower than that of cereals (Hadjischristodoulou, 1973). Studies in Syria have shown that rainfall explains half of the total variance of herbage yield, whereas temperature explains only a small part of the variance (Abd El Moneim et al., 1988). Bitter vetch is grown mainly during winter, under rainfed conditions in Mediterranean countries. It shows great fluctuation in seed yield (Turk, 1999), the main reasons being the sensitivity of the cultivars to different growing seasons or conditions. Information about the genotype (selection line) \times environment interaction and the stability of the genotypes is deficient in bitter vetch. Therefore, an attempt has been made to study some selection line \times environment interactions for seed yield, so that stable, superior lines could be selected for cultivation, as well as for inclusion in future breeding programmes. Therefore, the present study was an attempt to identify superior, stable genotypes of bitter vetch in relation to seed yield and 100-seed weight for semi-arid agroclimatic regions.

Materials and methods

Field trials were carried out at a field site on the Jordan University of Science and Technology (JUST) campus in northern Jordan (32° 34' N latitude; 36° 01' E longitude and 520 m altitude) during the 1996–97, 1997–98 and 1998–99 growing seasons. The JUST location typically experiences moderate to severe drought stress during the seed filling period. The soil at this site is smectitic, thermic, Typic Chromoxerert, very fine with a clay texture in the upper 15-cm soil surface. The pH in this horizon is 8.1, with an organic matter content of 16 g kg⁻¹ soil. Soil samples obtained from the upper 15 cm of the profile prior to the first year of the experiments were air-dried, processed and analysed for available P and N. Available P was 4.5 mg kg⁻¹ extractable with sodium bicarbonate and the available N was 1 g kg⁻¹ soil. In both years the plot area had been planted to barley during the previous cropping year. The experimental design was a randomized complete block with four replications. The plots were 4 m long and 2.4 m wide, with six rows, each with a row to row and plant to plant spacing of 40 cm. The treatments were nine *Vicia ervilia* selection (sel.) lines, obtained from the International Center of Agricultural Research in the Dry Areas (ICARDA): Sel. 2563 (L1), Sel. 2509 (L2), Sel. 2510 (L3), Sel. 2511 (L4), Sel. 2513 (L5), Sel. 2515 (L6), Sel. 2517 (L7), Sel. 2521 (L8) and the local check (L9).

The plots were sown by hand on 17, 15 and 18 November in 1996, 1997 and 1998, respectively, using a sowing rate of 80 kg ha⁻¹. Only one weeding was done, 30 days after sowing. The recommended rate of fertilizer was 100 kg ha⁻¹ of diammonium phosphate (N 18%, P₂O₅ 46%) applied as basic fertilizer at seedbed preparation. Supplementary irrigation of about 50 mm was added as necessary in each growing season.

Data on seed yield and 100-seed weight were collected from bulked seeds of ten randomly selected plants from each plot in each replication. The phenotypic index, i.e. the deviation of the overall variety mean from the grand mean, was determined. Stability parameters, i.e. the regression coefficient (bi) and deviation from regression (S² di), were obtained using the methods suggested by Finlay and Wilkinson (1963), Eberhart and Russell (1966), Perkins and Jinks (1968) and Freeman and Perkins (1971).

Results and discussion

The combined analysis of variance presented in Table 1 revealed that considerable genetic variability existed in the selection lines under inspection. The highly significant mean sum of squares due to environments for seed yield and seed index indicated differences between the environments and their considerable effect on these characteristics. The linear portions of the selection line × environment (L × E) interaction and the environment – (line × environment) interactions were highly significant, suggesting a considerable interaction of the genotypes with the environments for the characters studied. The magnitude of the non-linear component (pooled deviation) of the L × E interaction revealed that all the lines studied differed considerably in seed yield and seed index with respect to their stability.

The average performance of the lines in different growing seasons and their mean yield over all growing seasons with regression coefficients (bi) and deviations from regression (S² di) are presented in Tables 1 and 2. The highest seed yield (796.7 kg ha⁻¹) and seed index (4.83 g) were obtained in 1998–99, followed by 1997–98 (748.4 kg ha⁻¹ and 4.77 g) and 1996–97 (621.1 kg ha⁻¹ and 4.69 g).

Table 1

Average yield, phenotypic index (P), regression coefficient and deviation from regression of nine selection lines of bitter vetch evaluated in three successive years at JUST

Selection line	Origin	Seed yield (kg ha ⁻¹)			Mean	Phenotypic index (P)	Regression coeff. (bi)	Dev. from regr.(S ² di)
		96-97	97-98	98-99				
L1	Syria	527	652	634	604.3	-0.262	0.509	0.0206
L2	Cyprus	768	564	815	715.6	0.028	1.491	0.1859**
L3	Cyprus	329	531	446	435.3	-0.193	0.411	0.0323
L4	Cyprus	869	924	963	918.6	0.267	1.279	0.0069
L5	Cyprus	517	674	742	644.3	-0.008	1.541*	0.0019
L6	Cyprus	899	942	913	912.0	0.002	0.362	0.0631**
L7	Cyprus	1010	1042	1085	1015.7	0.146	0.981	0.0310
L8	Syria	696	713	846	751.6	0.019	1.436	0.0008
L9 (Local)	Jordan	675	694	727	698.6	0.022	1.429	0.0009
Environ. means		621.1	748.4	796.7	744.0			
Environ. index		0.790	0.226	0.559				
CV%		12.97	11.35	17.62	11.02			
SE		0.186	0.179	0.309	0.171			

Table 2

Average 100-seed weight (g), phenotypic index (P), regression coefficient (bi) and deviation from regression of nine selection lines of bitter vetch evaluated in three successive years at JUST

Selection line	Seed index (g)			Mean	Phenotypic index (P)	Regression coefficient (bi)	Deviation from regression (S ² di)
	96-97	97-98	98-99				
L1	5.09	4.88	4.91	4.96	2.72	0.623	0.2484
L2	4.87	4.92	5.11	4.97	5.54	0.754	0.5531**
L3	4.25	4.34	4.53	4.37	-2.56	0.731	0.0274
L4	4.66	5.08	4.51	4.75	0.74	1.002	0.7389**
L5	4.60	4.81	4.74	4.71	0.61	1.941**	0.2781
L6	5.14	5.24	5.61	5.33	1.86	1.025	0.2702
L7	5.62	4.61	4.74	4.99	-2.38	0.890	0.0502
L8	4.74	4.82	4.95	4.84	-0.28	0.803	0.0773
L9 (Local)	4.66	4.28	4.41	4.45	-1.46	0.785	0.0613
Environ. means	4.85	4.77	4.83	4.82			
Environ. index	0.81	0.19	0.46				
CV%	9.92	16.45	15.13	16.82			
SE	2.51	2.64	2.53	3.49			

This meant that the 1998–99 growing season was the most favourable environment and the majority of the genotypes had the capacity to exploit this environment to achieve the highest seed yield and seed weight. Though the highest yielding lines over all the growing seasons were L7, L4, and L8, they did not perform equally well in all the seasons (Table 1). The differences in yield of the lines in different environments indicated high reaction norms. Line L6 had the highest seed index (5.33 g), followed by L7 (4.99 g), L1 and L2

(4.96 g). An examination of the stability parameters b_i and S^2_{di} of the individual lines indicated that none of them showed combined b_i and S^2_{di} sensitivity, suggesting that either the non-linear component alone or their cumulative effects were responsible for the significant $L \times E$ interaction for seed yield and seed index. The lines which outyielded the grand mean yields were L8, L7, L6 and L4. Among the lines, L4, L8 and L9 had b_i values of more than one and S^2_{di} values around zero, suggesting that they were responsive to changing environments and could be recommended for favourable environments. L2 and L6 had significant S^2_{di} values; therefore, the prediction of these genotypes is not possible across environments. L7 had a b_i value near unity (0.981) and its deviation from regression (S^2_{di}) was non-significant, so this line was considered as the most stable one. The rest of the lines had yield performances below the experimental average. Most of them had b_i values below one, except L5. In general, it was observed that bitter vetch selection lines with high mean yields had less stability, i.e. yield and stability were inversely correlated. Banik et al. (1997) also reported similar results. In respect to seed index, among the lines studied, L5 had the highest b_i value (1.941) indicating the highest degree of responsiveness to changing environments (Table 2). On the other hand, L1 had the lowest b_i value (0.623), thus exhibiting the least change in 100-seed weight with the changing environments. L2 and L4 had significant S^2_{di} values. Thus, the prediction of these lines is not possible across environments. L6 had a b_i value near to unity (1.025) and a non-significant value of S^2_{di} . Therefore, it may be considered as the most stable line with respect to 100-seed weight. The rest of the lines had seed indices lower than the experimental average.

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Review

THE MULTIFUNCTIONALITY OF GRASSLANDS IN RURAL DEVELOPMENT IN A EUROPEAN CONTEXT

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Grasslands are the second most important land use systems on Earth. As natural resources they play a multifunctional role. The demand for grasslands is increasing and many new benefits of grasslands have been recognized in recent times. The European approach to rural development considers the 3 main functions of rural areas, and gives a definition of ruralism. European grassland systems, which involve extensive, moderate and high level grassland management, are characterized by sustainability. The paper discusses how EU policy influences grassland use in the member countries. The investigation of the potential of Hungarian grasslands in the context of the EU rural development approach outlines the high nature conservation value of species-rich native grasslands.

Key words: grasslands, rural development, grassland systems, nature reservation, biodiversity, amenity grasslands

Introduction

The grasslands of the world are managed in a changing environment as regards ecology, climate, world trading patterns, financial subsidies or incentives, and social demand. All these may have great influences on the new world concept, the sustainable management of natural resources. Recent developments in world globalization demands global thinking, which enforces scientists and policy makers to consider agricultural issues in their full complexity. Grassland management, however, is a practical matter, so besides theoretical global thinking by scientists, farmers acts locally. At this level farmers are facing many challenges originating from different social groups. These challenges are currently: market problems or overproduction in general, growing environmental awareness in the society, and growing social interest in nature reservation and biodiversity. All these, and the measures they bring about, enforce farmers to modify their practices to achieve a balance between different social interests.

In these contexts the purpose of this paper is to present a brief overview of grasslands in the world, the changing social demands for and benefits from grasslands, the European approach to rural development, the ways in which grassland functions and rural development objectives are matched in the European Union (EU), and the potential of Hungarian grasslands in rural development. Finally some conclusions will be summarized for public interest/consideration.

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Grasslands in the world

Area and ecology

In this paper the term grasslands is used to designate ecosystems in which the dominant vegetation component comprises herbaceous species (Coupland, 1979; Breymeyer and Van Dyne, 1980). In terms of land area grasslands are the second most important land use systems on earth, covering 3.354 million ha, which accounts for 25% of the total land area (Table 1). This area is more than twice that of arable land and is only 5% less than that of forests. The role of grasslands in land use differs from one continent to the other (Table 2). In Africa, Asia and Australia and Oceania grasslands are the most common land use systems, in the Americas and the former Soviet Union grasslands come second to forests, and it is only in Europe that the cropland area exceeds that of grasslands, indicating that Europe has the least grassland-focused agriculture.

The present situation is the result of a long land transformation history, which affected the extent and distribution of grassland areas in the world. Two trends characterized this long history. On the one hand large areas of the more fertile parts of natural grasslands have been converted into arable lands, while on the other hand forests have been converted into grasslands and pastures of various kinds (Buringh and Dudal, 1987). This tendency has not yet stopped. Recent restrictions on the extent of rural areas used for food production in Western Europe seem likely to result in shifts from cropland to pastures and from pastures to woodland (Hadley, 1993).

Table 1
Land area and use worldwide in 1991

Area and use	Million ha	%
Total area	14.041	100
Including		
Croplands	1.438	11
Grasslands	3.354	25
Forests	3.894	30

Source: WRI (1994–95)

Table 2
Land area and use according to continents in 1991 (million ha)

Continents	Cropland	Grassland	Forestry
Africa	181	900	684
Asia	457	759	531
North and Central America	271	362	709
South America	113	493	829
Europe	138	83	157
Former Soviet Union	229	326	827
Australia and Oceania	49	431	157

Source: WRI (1994–95)

Grasslands show the widest ecological amplitude of all the biomes on earth. For example, in Europe, they are distributed roughly between isotherms -1°C and $+18^{\circ}\text{C}$, being highly tolerant to extreme ecological elements such as frost, the permanent excess, fluctuation or deficit of underground water, flooding in winter and drought in summer. Grasses are also able to colonize the most extreme soils (peat, marsh, saline, low or high pH, etc.) (Rychnowská et al., 1994; Nagy, 1997).

Social demands for and benefits from grasslands

Over a long period of history grassland agriculture served as the major source of feed nutrients for domestic and wild animals. In this way grasslands played a key role in the development of civilization. In many countries and regions of Europe, for example, the principles of grassland management have been focused for centuries on the economically efficient production of good quality forage. As a result, animal yields and outputs per hectare have increased, but there have often been negative effects on landscape value and on the diversity of wild flora and fauna (Green, 1990). These developments have now resulted in a sophisticated grassland philosophy, which underlines the multifunctional role of grasslands in modern societies. The present demands for and benefits from grassland are as follows:

- to provide livestock with good quality forage,
- to furnish a habitat for wildlife, both flora and fauna,
- to contribute to the attractiveness of the landscape,
- to provide suitable vegetation cover for outdoor recreation and pleasure.

Beyond these socio-cultural aspects of grassland use, grasslands, as natural resources, must also be evaluated on a global scale. In this respect some of the benefits of grasslands as natural resources should be mentioned. It is obvious that grasslands have the greatest potential in soil erosion control and in improving soil structure and fertility. Grasslands can contribute to the stabilization of the gas components of the atmosphere to a large degree by fixing CO_2 and releasing O_2 . It has also been recognized that grasslands can play a key role in water conservation, water resource development and protection. It is impossible to overestimate the contribution of grasslands to the improvement and protection of the environment from pollution such as sediment, windblown soil, municipal and farm wastes and certain toxic substances (Láng, 1994; Barnes and Baylor, 1995; Nagy and Vinczeffy, 1993). As new technologies become available, it will be possible to utilize the complementary benefits from grasslands by converting grass biomass to energy as a renewable resource, or utilizing potential sources of high quality extracted leaf proteins and other plant products from forage and herbs produced on grasslands.

The future of grassland use on a global scale is likely to show a diversified picture. There can be no doubt of the continuing importance of grasslands in food production and environmental stability, and it seems probable that there

will be continuing emphasis on relatively simple pastoral systems in most regions of the world and a move away from high capital, high-energy systems in regions where they currently exist (Hodgson, 2001). The multifunctions of grasslands will be developed continuously as social demands arise and new technologies become available in the new millenium (Nan, 2001). As in the past (Nösberger et al., 1994) it will be possible in the future to integrate the objectives of grassland use, but this needs policy support and has to be accompanied by compensation schemes for farmers.

Classification of grassland systems

The existing grassland systems are determined by two main factors: ecological (climate, soil) and socio-economic conditions (habitat of population, agricultural policy, level of development, etc.). Grassland system categories can be set up from different points of view. From the point of view of origin, grasslands include planted pastures as well as a variety of natural rangelands in situations ranging from semi-arid to subhumid conditions (Hadley, 1993). Planted pastures or grasslands are also referred to as cultivated grasslands and these characterize grassland farming in some Western European countries (Frame, 1992). The target-oriented view of grassland use employs the categories of foraging grasslands and special purpose grasslands (Mannetje, 1994). Diversity in grassland systems is also reflected in the spectrum of grassland users and uses, for example nomadic pastoralism, extensive ranches, intensive pastures, wildlife tourism and game hunting (Hadley, 1993). As regards the level of intensity (Nagy, 1997) high input, lower input (moderate level) and extensive grassland systems can be distinguished. The vast majority of the world's grasslands are extensive. According to scientific estimations (Buringh and Dudal, 1987) only about 500 million ha of grassland (i.e. a sixth of the world total) are in the high and medium land use categories, with five-sixths in the low and zero classes. It should be noted that the level of intensity at which the grassland is managed also indicates the interactions between grassland and environment, which deserves great attention if sustainability is the main consideration of farming. Below, the three main grassland systems of Europe will be briefly evaluated from the point of view of sustainability.

Grassland systems in Europe

Extensive grassland systems

These systems prevail, at least in Europe, under unfavourable ecological conditions: high mountains, steep slopes, dry climate, poor soil conditions, areas with a high water table, temporarily flooded wetlands, or areas where environmental regulations or measures do not make it possible to farm grasslands more intensively.

These systems are free from all the harmful effects that may threaten the sustainability of the environment. The grassland management is characterized by very low inputs, sometimes as low as zero, and farming is aimed at the utilization of the biomass. There is no surplus nutrient budget and there are no extra gas emissions to the environment due to fertilization inputs.

Because of the nature of these systems, extensive grasslands (at least in some parts of Europe) are simultaneously of great value for nature conservation, whilst semi-natural grasslands provide the main vegetation types, next to forests, in nature reserves (Mannetje, 1994).

Grassland systems with a moderate level of inputs

These grassland systems are associated with the use of clovers, mainly white clover, in seed mixtures for the renovation or establishment of productive grass swards. The reason for the use of clovers is that farmers can reduce N inputs on their grasslands, as clovers fix substantial quantities of N from the atmosphere into the soil. At a high technical level of production, this system is comparable to the high input systems and may produce similar yields of milk (Bax and Schils, 1993) or beef (Mould et al., 1993) per hectare. The weak point of the system is at present the unbalanced availability of fixed N during the vegetation period (Wilhelmy and Kornher, 1993). The main advantage of biologically fixed N is that it does not cost fossil fuel. Unfortunately, the high rate of N fixed may result in excessive N emissions into the environment (Elgersma et al., 1993; Scholefield and Tyson, 1992).

High input grassland systems

These systems, frequently referred to as intensive systems, exist in certain developed countries in temperate regions, e.g. Western Europe. These systems are practised mostly on dairy farms. Farmers use high rates of N fertilizers and/or slurry, which may reach 400–500 kg N ha⁻¹. They mostly graze the herbage produced, the rest being preserved as silage or hay.

When examined in terms of sustainable agriculture, the system raises several questions. The main problem is that overall efficiency of nitrogen, in terms of milk and meat production, is generally less than 20%. Under grazing conditions, a high percentage is returned to the soil as dung and urine. When the grass crop is harvested by cutting or conservation, the nitrogen is ultimately recycled through the slurry of winter housed livestock.

When high levels of fertilizer nitrogen (e.g. 400 kg N ha⁻¹) are applied to pastures on freely draining soils that are grazed by beef cattle, in the short term, 15–30% of the N is lost by volatilisation as NH₃ into the groundwater. In the case of heavier soil conditions, with impeded drainage, losses due to leaching are reduced, but denitrification losses increase (Garret et al., 1992).

Similar trends can be observed with other macroelements. The conversion rates for different elements, defined as nutrient output via animal products as a percentage of total nutrient input into the farms, show a relatively low

conversion of applied nutrients (Aarts et al., 1988). The rates have been found to be about 13–14%, 29–33% and 13–19% for N, P and K, respectively, on different soil types (Table 3). As a result there is a high annual surplus of N, P and K applications (Table 4) in high input grassland systems (Weissbach and Ernst, 1994).

The system can also be investigated according to the emission of greenhouse gases. Emissions include N_2O and CH_4 from biological resources within the grassland agriculture system (soil, rumen), CO_2 emissions from burning the fossil fuels used to drive farm machinery and to produce fertilizer, and other gas emissions from external sources that are associated with grassland systems (Mannetje, 1994).

Table 3

Mean nutrient surplus and conversion rates of 996 specialized Dutch dairy farms during the years 1983–86 (Conversion rate is defined here as nutrient output via animal products as a percentage of total nutrient input into the farm)

	Soil	Plant nutrients		
		N	P	K
Surplus $\text{kg ha}^{-1} \text{yr}^{-1}$	Sand	487.0	32.0	125.0
	Clay	466.0	33.0	78.0
	Peat	462.0	30.0	94.0
Conversion rate %	Sand	14.5	32.9	13.9
	Clay	13.3	29.6	19.0
	Peat	13.5	31.5	16.7

Source: Aarts et al. (1988)

Table 4

Average annual nutrient balances in 1983–1986 for about 175 specialised dairy farms on sandy soils in the Netherlands

	Nutrient ($\text{kg ha}^{-1} \text{year}^{-1}$)		
	N	P	K
Inputs:			
artificial fertilizers	331	15	30
purchased concentrates	137	25	74
purchased roughage	44	6	34
atmospheric deposition	48	1	4
miscellaneous	8	1	4
Total	568	48	146
Outputs:			
milk ¹	67	12	19
sold livestock ²	14	4	1
sold roughage	1	0	0
Total	82	16	20
Inputs–Outputs	486	32	126

¹ about $13,000 \text{ kg/ha}^{-1}$, ² about 540 kg^{-1} , Source: Aarts et al. (1992)

A comparison of low and high intensity dairy farms in this respect (Bakken et al., 1994) has shown that the "global warming effect" of all emissions (CH_4 , N_2O and CO_2) was $9.1 \text{ t ha}^{-1} \text{ year}^{-1} \text{ CO}_2$ equivalent for a high intensity Danish dairy farm and $6.5 \text{ t ha}^{-1} \text{ year}^{-1} \text{ CO}_2$ equivalent for a low intensity farm (Table 5).

Both nutrient budgets and gas emissions have to be given special attention when discussing the sustainability of high input grassland systems.

Due to the negative effects of intensive systems on the environment there is a move in industrialized countries to convert areas of intensive production grassland into more diverse vegetation types, which is of special interest to nature conservation (Mannetje, 1994).

Table 5
Global warming effects of the various sources of gas emissions from grassland farming
(expressed as t CO_2 equivalent year^{-1})

Gas type	HI farm		LI farm	
	ha^{-1}	total	ha^{-1}	total
N_2O	1.8	67	1.0	78
CH_4	5.0	185	4.1	319
Energy	2.3	85	1.4	109
Total	9.1	337	6.5	507

HI = high intensity, LI = low intensity, Source: Bakken et al. (1994)

The European approach to rural development

Rural development is a new concept in European socio-economic development. Until the end of the last programming period (1993–2000) the main EU policy measures which focused on rural areas were included in the Common Agricultural Policy (CAP) which dealt with agricultural issues in a narrower context. However, policy makers have recognized the differences in the level of development between rural and urban areas, forecasting that in a speeded-up development process rural areas will lag behind urban ones. This realization has led to agriculture being put in a wider context, which in this programming period (2000–2006) is called CARPE (Common Agricultural and Rural Policy for Europe). The term was taken from a report by Buckwell (1997). Representatives of development and territorial sciences usually refer to the same initiatives when they look for the development of this new rural policy of EU.

The European Chart of Rural Areas (Anonymous, 1995) brought a change in thinking on rural problems. It raised rural problems to the level of top EU policy. For the first time the idea of value was expanded to include not only material and financial aspects but also non-material elements such as cultural, community and natural values. The Chart underlined the multifunctional role of rural areas and gave them integrated functions:

- economic functions (use of natural resources; agriculture; forestry; food processing; services; trade; very diversified economy in general),
- ecological functions (protection of land, water, atmosphere; preservation of wildlife; maintaining biodiversity; protection of habitats; landscape development)
- socio-cultural functions (maintaining rural life-style; small community life; folklore; cultural heritage; responsibility for civilized landscape; provision of recreation facilities for urban people through rural tourism, etc.).

In 1996 the Cork Declaration (Anonymous, 1996) was in favour of "a living Countryside", saying that society should be "aware that rural areas, which are the home of a quarter of the population and account for more than 80% of the territory of the European Union, are characterized by a unique cultural, economic and social fabric, an extraordinary patchwork of activities, and a great variety of landscapes (forests and farmland, unspoiled natural sites, villages and small towns, regional centres, small industries)".

The European concept of rural areas has now been defined, stating that an area should be considered as rural if

- agriculture is the dominant activity of the local community,
- green vegetation (forests, grasslands, croplands, vineyards, orchards) covers a large proportion of the ground surface,
- settlements are small,
- the ratio of built-up areas is low,
- the population density is relatively small.

Finally, the concept of rural development should be defined. From the above it follows that rural development is concerned with activities with integrated, multifunctional, multidisciplinary or complex nature, which will enable rural areas to fulfil their important functions for the benefit of present and future generations.

Matching grassland functions and rural development objectives in EU policy

The present EU policy helps to develop a genuinely multifunctional, sustainable and competitive agricultural sector, which will also help to secure the future of the more vulnerable rural areas. It recognises that agriculture has a key role to play in preserving the countryside and natural spaces and in the vitality of rural life.

The rural development policy is also considered essential to agriculture, a sector which is confronted by a fresh series of challenges, including environmental considerations. Farmers must rely on a living countryside, so opportunities should be given for them to diversify their activities. This will be beneficial not only for them, but also for their families and for the local community. Ultimately, society as a whole will gain from a more prosperous rural Europe (Anonymous, 1997).

It is already broadly accepted by EU member countries that rural development is essential for the environment and the quality of life. In this respect it is very important to provide a natural environment, environmentally attractive living or recreational spaces, and grasslands for amenities and leisure. For example a survey in the UK estimated that amenity grasslands cover about 4% of the country (Table 6). These grasslands improve the quality of life in many ways, in the form of sportsfields (golf, cricket, soccer, etc.), school playing fields and lawns, nature reserves, domestic lawns, caravan and picnic sites, river banks, etc. (Green, 1989).

The present agreement on EU agricultural and rural policy contains elements which may influence grassland farming in direct or indirect ways in Europe. The extent to which the policy influences a country, however, depends largely on the role of grasslands in land use and the strength of animal production industries based on grasslands. The proportion of grasslands in agricultural lands ranges between 28 and 67% in some EU countries, which is much higher than that in some Eastern Central European countries (Table 7). In some EU countries (The Netherlands, UK, Ireland, Denmark, Belgium, Germany, Austria, etc.) the very strong dairy and beef industries are based on intensively managed grasslands. The philosophy of a "greener CAP" is aimed at the extension of compensatory allowances in support of farming in less favoured areas (LFAs) to areas where farming is restricted by the existence of specific environmental restrictions.

These specific environmental restrictions affect grasslands on nature reservation areas. Farming systems with high nature value are encouraged, including livestock farming (sheep, goats and beef cattle), especially on semi-natural grasslands, lowland wet grasslands, moorland and heaths, mountain pastures, wooded agri-pastoral lands and Mediterranean scrubs, or dairy cattle in some regions, such as grazing of mountain pastures and coastal marshes (Anonymous, 1997).

The potential of Hungarian grasslands in rural development based on the European approach

The proportion of grasslands in Hungarian agriculture is far lower than in the EU (Table 7). Their role in landscape development differs from one region to the other. In some areas grasslands are the most common land use system, giving the landscape an open appearance.

The economic function of Hungarian grasslands, unlike the EU situation, is negligible for several reasons:

- land transformation from grassland to cropland was continued up till the last decade of the past century. Grasslands have remained only on soils not suitable for arable cropping. Soil fertility is extremely low on these marginal soils, which produces only about 1.5 t dry matter ha⁻¹ year⁻¹ on average.

Table 6
Amenity grass categories and areas in UK (1973)

Categories	Area (km ²)
<i>Intensively managed areas</i>	Approx. 1100
School playing fields and lawns	490
Armed services sports pitches and lawns	70
Other football and hockey pitches	90
Golf fairways	348
Golf greens	17
Golf tees	9
Cricket grounds (privately owned)	62
Bowling greens	5
Horse race tracks	5
Greyhound tracks	0.2
Field sports stadia	(No separate figures)
Tennis courts	(No separate figures)
<i>Trampled open spaces</i>	Approx. 2700
Man-made	
Urban parks and open spaces	1345
Domestic lawns	900
Urban and suburban road verges	250
Armed services sport outfields	190
Semi-natural	
Rural road verges	1010
National Trust land	922
Nature reserves	630
'Common land'	526
Golf 'rough'	497
Miscellaneous County Council land	300
Caravan and picnic sites, nature trails	70
River banks	49
Country parks	60
<i>Untrampled open spaces</i>	Approx. 630
Military airfields	259
Railway embankments	202
Civil airports	110
Motorway embankments	56
Dam faces	0.8

Table 7
Grasslands in land use in some EU and Eastern Central European countries

Country	Grassland as a % of agricultural land
United Kingdom	67
The Netherlands	61
Austria	56
France	38
Germany	31
Italy	28
Czech Republic and Slovakia	24
Poland	22
Hungary	18

- Production inputs are extremely low on Hungarian grasslands (Table 8). Fertilization, irrigation, improvement or renovation and weed control are all negligible in grassland management, contributing to the very low output of grasslands.
- Due to the foraging system in the country the ruminant industry is mostly based on arable crops, so grasslands are utilized at a low level. It has been estimated (Nagy and Pető, 1995) that only 1/3 of the grasslands is regularly utilized, 1/3 is temporarily utilized and the last 1/3 is not utilized at all.

Table 8
Inputs on grasslands (Large farms), 1996

Fertilized area (%)	5.2
Fertilization (kg ha ⁻¹ on total area)	3.6
Fertilization (kg ha ⁻¹ on fertilized area)	68.8
Herbicide application area (%)	0.2
Fungicide/pesticide application area (%)	0.3

Source: NSA (1996)

In contrast with the economic function, Hungarian grasslands, as rural areas, have great ecological potential. They play a very important role in land use on nature protection areas (Table 9). After forests, grasslands are the second most important land use system on protected areas. In fact, on the most strictly protected areas (national parks, nature protection lands) the role of grasslands is comparable with that of forests.

Table 9
Types of land use on nature protected areas (ha)

Protected areas	Cropland	Grassland	Forest	Others	Total
National parks	14.304	66.247	63.558	33.629	177.738
Landscape protected areas	68.362	94.567	252.592	51.132	466.653
Nature protected lands	2.451	7.256	11.028	5.225	26.230
Total (ha)	85.127	168.340	327.178	89.986	670.621
(%)	12.7	15.1	48.8	13.4	100.0

Due to the very low production inputs and the low level of utilization the nature conservation value of Hungarian grasslands is outstanding, as changeable ecological conditions in the Carpathian Basin have created species-rich ecosystems. For example, the most common Hungarian grassland associations contain more than 40 plant species on average (Table 10).

Table 10
Features of some frequent grassland types in Hungary

Number of evaluated grassland types	Level of water supply %			Average species number (extreme values)	Average quality ranking (min. 1; max. 5)	Average yield t ha ⁻¹ DM in natural stage
	good	moderate	poor			
36	36	35	39	42.4 (11–66)	2.2	1.5–2.5

After Vinczeffy (1993)

Many of the species found on grasslands have become endangered due to human activities (water control, agricultural production, narrowing wildlife habitats by extending the infrastructure). In a recent study (Nagy, 2001) it was found that 45% of the protected species described in the Hungarian Red Book require grassy vegetation (Table 11).

Finally, Hungarian grasslands also have great potential in the socio-cultural functions of rural areas. The heritage of pastoralism (folk dances, songs, folk arts, e.g. clothes, crafts, musical instruments) in rural areas has high cultural value which is worth preserving. Unspoiled grassy rural landscapes have a national role in providing recreation and leisure for urban society through rural tourism, eco-tourism or game hunting. Many of the medicinal and melliferous plant species growing on Hungarian natural grasslands (Vinczeffy, 2001) could contribute to the re-development of natural medicine.

Conclusions

Grasslands are vast natural resources, the use of which depends on ecological and socio-economic conditions. There are new challenges facing grassland use, including climate change and new social demands. Among the latter, concerns about environment and nature reservation, the quality of the landscape, and the suitability of open spaces for recreation are increasing.

Table 11
Role of Hungarian grasslands in maintaining protected animal and plant species*

	No. of species demanding grassy vegetation	No. of species not demanding grassy vegetation
ANIMALS		
1. Vertebrates		
Mammals	3	9
Birds	36	33
Reptiles	3	1
Amphibians	—	1
Vertebrates in total	42	44
2. Invertebrates		
Snails	—	8
Insects	95	133
Invertebrates in total	95	141
Animals in total	137	185
Proportion of two animal groups	43%	57%
PLANTS		
Angiosperms	26	14
Pteridophytes	—	1
Plants in total	26	15
Proportion of two plant groups	63%	37%
ANIMALS AND PLANTS IN TOTAL	163	200
Proportion of two species groups	45%	55%

* Existing species described in the Hungarian Red Book (1989) in detail; Source: Nagy (2001)

The rural development policy of the EU underlines the multifunctional role of rural areas. New measures introduced in this context in the EU may influence grassland use in several ways. Environmental limits will be imposed on intensive use, while extensive use will be encouraged to promote biodiversity and a more attractive landscape.

The present economic role of Hungarian grasslands in the development of rural areas is negligible. However, they have great potential in nature reservation, which offers opportunities for the improvement of rural life through the development of tourism.

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Book reviews

A. ERDÉLYI (2001): *The man who harvested sunshine. The modern Gandhi: M. S. Swaminathan*. Tertia Kiadó, Budapest

I have been acquainted with Professor M. S. Swaminathan (hereafter M.S.S.) for the last 30 years. We first met at mutation conferences, and I have since followed his career with great interest. In lectures on wheat, making up part of a course on tropical breeding, I dealt in detail with the Green Revolution, making special mention of the results achieved in India by M.S.S.

In 1948 the total wheat yield in India amounted to 4 million tonnes. In 1956 M.S.S. obtained semi-dwarf wheat varieties with strong straw from N. Borlaug, the wheat breeder working in Mexico, and crossed these with Indian varieties. Using varieties arising from these crosses, or using the Mexican varieties themselves after selection for adaptation to the ecological conditions, Indian farmers harvested 4–5 t/ha in place of the previous 1 t/ha. Naturally, this also required the development of cultivation techniques suited to local conditions. As a result of this work, India produced a total of 72 million tonnes of wheat in 2000 and from being a wheat importer, became a wheat exporter.

After such preliminaries, I picked up the book written by András Erdélyi with great interest and found it to contain a great deal of new information both on the professional and organisational work of M.S.S. and on India. The professor's wife also deserves our admiration, not for her genetic research, but for her efforts on behalf of village women, who bear a far heavier burden than the men both in tilling the land, in the household and in raising their children. Her realistic judgement on the continuation of the caste system is astoundingly true.

The author is to be congratulated on his presentation of the Buddhist way of thinking and of the heritage left by Gandhi, since these are necessary if the professor's life-work is to be appreciated. The essence of this is that an improvement in the situation must start with the very poorest and most destitute and must continue gradually from

there. This is the special role and responsibility of the scientist.

The book, which takes the form of conversations with M.S.S., goes on to discuss how he began his research career, and what progress has been achieved in easing the work of villagers. The scientists know they must produce something new each year if they are to retain the respect of the farmers. They have had to breed not only new wheat varieties, but also new breeds of buffalo for use in cultivating the fields. Their work is based firmly on traditions and realities and is aimed at improving ecological methods of farming.

It may come as a surprise to find that a wheat breeder is involved in saving the coastal mangrove forests and in environment protection. These forests, however, protect the arable land, and even in India the role of the sponsor in the choice of research topics is not unknown.

The author writes well and is well versed in his subject. He provides a great fund of knowledge on the human and professional merits of M.S.S. I should like to congratulate him on this volume and hope he will write further books on other Hungarian or foreign scientists.

A. BÁLINT

Környezet- és Természetvédelmi Lexikon. (Encyclopaedia on Environment Protection and Nature Conservation) Vols. 1–2. Editor-in-Chief: ISTVÁN. LÁNG. Akadémiai Kiadó, Budapest, 2002

The first edition of this Hungarian encyclopaedia published in 1993 under the title *Environment Protection Lexicon*, contained 8000 entries. During the eight years which passed between the first and second editions, very significant changes were witnessed in the field of environment and nature protection, both in Hungary and worldwide. It is enough to mention the two major world conferences on environment protection, held in Rio in 1992, when the first edition was prepared, and in Kyoto in 1997. Environment protection is gradually becoming an integral part of our everyday lives, influencing the economy, rural development, politics ("green" parties) and the legal system. Knowledge on environment protection is

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playing an ever greater role at all levels of education.

Environment and nature protection is already an integral part of the legal system of the European Union, and these laws will also become binding in Hungary when the country becomes a member state.

There is thus every justification for this new, second edition of the lexicon, the new title of which (Encyclopaedia on Environment Protection and Nature Conservation) is indicative of a change in attitude and content, which is also reflected in the number of entries.

The two volumes contain a total of 9500 entries written by 354 authors. The excellent quality photographs were prepared by 48 photographers. The first volume (A–K) consists of 664 pages and the second (L–Z) of 558. Many of the entries are illustrated with figures or maps prepared by the Cartography Department of Eötvös Loránd University and by the Nature Protection Office of the Ministry of Environment Protection. The only possible objection is that in some cases the photos and figures are rather small, but this was obviously necessitated by restrictions on the size of the encyclopaedia.

It would be impossible to list all the authors in a short review, but mention should be made of some of the better known: Ferenc Glatz, Gábor Karátson, György Füleky, László Hornok, Zolt Harnos, László Heszky, István Láng, Ferenc Ligetvári, Imre Loksa, Tibor Simon, Pál Stefanovits, János Tardy, György Várallyay and Tamás Vásárhelyi. These names will be sufficient to give some idea of the wide range of subjects to be encountered in the lexicon.

The following selection will give an indication of the multiplicity of the entries, the first of which is the Aarhus Agreement and the last the Zwikler method for calculating loudness: American nature protection areas, sewage cleansing, respect for life, photoelectric

energy transformer, Gene Technology Law, Long Term Ecological Research Network, industrial waste, IUCN endangered categories, Environment Education and Communication Programme Office, ecological architecture, precision farming, acid rain, single issue organization, saline soils, nature protection fines, Tropical Forest Action Plan, greenhouse effect, WWF.

Various appendixes contain lists of Hungarian and foreign nature protection publications, Hungarian colleges providing courses on environment protection, and the major organisations in Hungary dealing with environment and nature protection. The plants and animals granted protection or strict protection in Hungary and the EU are listed in Hungarian and Latin.

The importance attached to this enterprise is best indicated by the fact that the editorial committee was set up jointly by the Hungarian Academy of Sciences, the Ministry of Environment Protection and the Parliamentary Committee for Environment Protection. The publication of the encyclopaedia was sponsored by numerous ministries, local councils, foundations and major firms such as MOL (Hungarian Petroleum), Richter Gedeon Co. Ltd. and Béres Co. Ltd.

In addition to the printed version, the encyclopaedia is also available, at greater length, on CD-ROM, and the publishers also plan to make a constantly updated version available on the Internet.

The encyclopaedia can be recommended to all those who are interested in the protection of the environment. It will prove a useful handbook for teachers and students, and it is a must for all public libraries and for scientific, school and university libraries. Hopefully it will be of assistance to the younger generation in remedying the environmental damage caused by the generations who went before them.

B. KÓSZEGI

CEREAL RESEARCH COMMUNICATIONS

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INTEGRATION OF TRANSFORMATION TECHNOLOGY AND TRADITIONAL BREEDING OF CEREALS

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The integration of conventional plant breeding and plant transformation is necessitated by the fact that, prior to the gene technological phase, traditional breeding methods have to be used to develop agronomically valuable homozygous genotypes which can then be modified for a gene or genome segment by means of gene manipulation techniques. Once the genotype selected by means of conventional breeding has been transformed, traditional methods are again used to examine the agronomic properties of the lines developed from the transgenic plant and the stability of the transgenic variety, following the DUS criteria elaborated by UPOV. The seed production of genetically modified plant varieties must be safe and economical and the cultivation of the variety should contribute to the sustainable development of up-to-date crop production.

Key words: wheat, breeding, transgenic variety, transformation technology, agronomical performance, HMW glutenins

Introduction

During the 5000-year history of plant breeding there has never been such an enormous change as has been observed over the last twenty years, with the appearance of transgenic plant varieties developed by means of biotechnology. Due to the results achieved over this relatively short period, at the turn of the millennium every third hectare of soya, every seventh hectare of cotton, every ninth hectare of rape and a similar proportion of the maize grown in the world involved genetically modified plants (James, 2000). This period was the logical continuation of the green revolution in the second half of the last century, which effectively countered starvation by a quantitative increase in plant productivity and intensive farming on industrial lines. At the same time, limits to quantitative development became perceptible in developed countries (Table 1), since the increase in the yield potential of cereals slowed down all over the world in the nineties compared to that achieved over the previous four decades (Brown, 1998). These changes affect the whole of agriculture and involve the need for

- sustainable growth in crop production
- a broader emphasis on quality improvement instead of quantity criteria
- priority setting to achieve an ecological equilibrium
- an increase in food safety and security.

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Table 1
Annual change in world grain yields by decades as a % (1950–1995)

Year	Total grain	Rice	Wheat	Maize	Other grains
1950–60	2.0	1.4	1.7	2.6	
1960–70	2.5	2.1	2.9	2.4	2.3
1970–80	1.9	1.7	2.1	2.7	0.4
1980–90	2.2	2.4	2.9	1.3	1.7
1990–95	0.7	1.0	0.1	1.7	–0.8

Source: Brown (1998)

All these tasks represent a new challenge for plant breeders, and a solution is only likely to be found if conventional and molecular breeding methods are integrated. The search for new solutions will serve to ensure the sustainable development of farm production.

Aims of integrated plant breeding

The aim of molecular plant breeding is to use gene manipulation techniques to induce beneficial changes which cannot be achieved at all, or with far lower efficiency, by means of traditional breeding. By transforming cereals it is possible to increase the agronomic performance of the plant, improve the efficiency and stability of production, and broaden the end uses of the crop. One facet of molecular breeding is the use of plant transformation for breeding purposes, which could be a useful tool in developing the multifunctional agriculture demanded by society, where, in addition to the cultivation of plant products, priority is also given to preserving the ecological equilibrium, to rural development and to promoting healthy nutrition. Molecular breeding, including the transformation technology, could thus be of great importance not only in high input or precision farming systems, but also in the establishment of low input, sustainable agricultural systems. The aim of integrated plant breeding is to apply a mixture of molecular breeding and traditional breeding methods in order to develop plant varieties which can be grown under various crop production conditions. Problems awaiting a solution include

- the need to reduce pesticide pollution in ecologically sensitive regions, for example by breeding genotypes resistant to herbicides, fungal and viral diseases, or insects;
- the improvement of yield stability, for example by breeding genotypes with cold, drought or salt tolerance;
- the production of foodstuffs promoting healthy nutrition, for example by increasing the vitamin content, improving plant nutrient transport or making plants produce essential amino acids;
- improvements in the quality of life, for example by producing the macromolecules required by the pharmaceuticals industry on organic farms or by reducing the number of allergens.

The breeding of transgenic plants is aimed to an ever greater extent at quality improvement and at reducing the environmental damage caused by farming, while an increase in yields is becoming less and less important. This is confirmed by a survey carried out by USDA in 1999, demonstrating that the yield advantage of maize and cotton containing the Bt gene or of Roundup ready soya was only 4.4–10%, while the use of these crops led to a reduction of 22–90% in the use of herbicides and insecticides compared to traditional technologies, proving its environmental benefits unambiguously (Birch, 2000).

Plant breeding is always designed to increase genetic diversity and to utilise this in new plant varieties. Now that plants can be analysed at the DNA level new dimensions have opened up in plant breeding. These include

- the availability of complete genome sequences
- the identification of agronomically important candidate genes for transformation using microarray technologies
- the characterization of cereal genebank collections at the DNA level
- the introduction of alien genes or large blocks of genome to widen the diversity of the cereal gene pool.

Transformation technology and plant breeding

Gene technology is just one phase in the development of a new plant variety. From the plant breeding point of view the following are required for the development of a transgenic variety:

- the isolation of a gene or gene section from the donor genome for the purpose of transformation
- a homozygous plant or target genome developed by traditional breeding and suitable for transformation
- a transformation protocol with a suitable promoter for the development of a transformed plant
- the development of lines with breeding value from the transformed plant, leading to the development of a transgenic variety

The development of a transformed plant is not identical with the development of a new transgenic plant variety. It is here that the need to integrate conventional and molecular breeding methods arises, since, prior to the molecular phase, an agronomically valuable, homozygous genotype must be developed by means of traditional breeding, which can then be modified through the incorporation of a gene or genome section by means of gene manipulation. After the transformation of this genotype, itself selected in the course of conventional breeding, traditional breeding methods will again be used to select for a transgenic plant variety, which must meet the following criteria:

- it must have a stable genome, stably inherited in later generations and thus conforming to the DUS criteria laid down by UPOV;
- due to the stable expression of the alien gene, combined with its other traits, the transgenic variety should be agronomically suitable for introduction into cultivation;
- its flowering biology characteristics should be stable, so that seed can be produced safely and economically;
- it should give stable yields within the production region without environmental risks;
- the incorporation of the transgene should provide an economic advantage over the donor variety, making the new variety commercially valuable to the breeder.

Molecular breeding has introduced new technologies into plant breeding. It is likely that in the future each laboratory will produce thousands of transformed plants, but only a fraction of these will be of value in breeding. Again only a fraction of the transformed plants with breeding value will possess the economically and commercially useful traits required if they are to satisfy the criteria raised for a transgenic variety.

The use of the transformation technology will be effective if it allows plant breeders to meet the demands of society more efficiently. This will require the elaboration of a technology which can be used routinely by breeders. At present, however, there are still considerable differences between the various cereal species in this respect. While the transformation of rice can already be achieved fairly routinely, common wheat and durum wheat are difficult to transform for breeding purposes due to the substantial genotype dependence. For plant breeding purposes it will be essential to develop and apply a transformation system which is largely independent of the genotype. The efficiency of the transformation technology from the breeding point of view depends on many factors. It will only be ideal if gene transfer leads to a stable transformation which does not affect the other breeding and agronomic traits of the genotype. At present two gene transfer systems are generally applied:

- Direct gene transfer (DGT) techniques (cell or tissue electroporation, microinjection, particle bombardment, etc.), among which particle bombardment is the most frequent;
- *Agrobacterium*-mediated transformation, which is still in the experimental phase for several cereal species (e.g. common wheat) in most laboratories, but promises to be a simpler, more efficient technology.

Transgenic cereal varieties can be used directly in breeding if the variety is suitable for direct use as a transformation target genome. This is only possible if the variety can be easily transformed. However, in many cereal species the genotypes or varieties are not all equally suitable for transformation for breeding purposes. Where there is great genotype dependence, as in the case of common

wheat, it is advisable to start with the most easily transformed genotype and to employ the backcross method to transfer the gene into agronomically important varieties.

One basic criterion for planned molecular plant breeding is the successful incorporation of the transgene into the desired plant tissue. At present, however, this is largely fortuitous due to the randomly distributed integration sites. It appears likely that the recipient genomic DNA is only integrated with the alien DNA if there are short partially homologous regions, but the mechanism of this is still not completely understood. It is, however, clear that a repair process takes place at the point of contact between the alien and genomic DNA.

Due to the weight of public opinion, it is essential for plant breeders to give special attention to the alleged or real environmental risks involved in the use of transgenic plant varieties. These include the herbicides and antibiotics used as selection markers. Although no scientifically-based evidence is yet available in this connection, due to the potential danger it is advisable to avoid the application of these compounds, especially when developing transgenic plant products for direct human consumption. Numerous techniques are available to solve this problem, such as

- the removal of the selection marker genes by natural means
- the application of technologies free of marker genes
- the use of positive marker selection systems (e.g. the mannose selection system).

The development of tissue culture methods was already fairly advanced before transformation research was begun. The choice of the best regeneration system for the transformed plant depends on the type of transformed explant, which may differ from one species to the other in the case of cereals.

The selection methods used in transgenic variety breeding differ considerably depending on whether plant regeneration is induced from diploid or haploid cells. In the case of transgenic plant regeneration from somatic cells a selection system is required which will lead to the selection of a transgenic, homozygous genotype. It is simpler for breeders if the regeneration protocol involves the use of haploid cells, from which homozygous transgenic plants can be raised after rediploidisation, thus saving both time and expense.

When employing tissue culture methods, problems are still encountered due to the genotype dependence of certain species. One indirect solution to this problem is to use a transformation system involving a model plant which can be easily transformed and regenerated. The gene incorporated into the model plant then has to be transferred via the backcross method, thus increasing the time required for the development of an agronomically useful transgenic variety.

The achievement of transformations which will be of use in breeding is greatly influenced by the promoter applied. This is particularly true in the case of monocots, including cereals, since some promoters are less effective in monocots than in dicots. The promoter may have a decisive influence on the

stable expression of the incorporated gene and on the extent to which the desired agronomic trait is expressed. Breeders must also be aware of the environmental risks represented by the promoters, even if these risks are only imaginary, since this may have a substantial influence on the public acceptance of the variety. For this reason, considerable thought must be given as to whether constitutive, selective or inductive promoters should be used in the gene technology. Limited availability due to legal protection may also influence the choice of promoter.

Selection of transformed genotypes for the development of transgenic varieties

Transgenic plants in themselves do not satisfy the criteria to be met by plant varieties, so they must be subjected to a selection process prior to practical introduction. One of the most important criteria is the stable expression of the transgene in the progeny generations. Selection serves to identify homozygous transgenic genotypes in the segregating generations, to develop lines where the transgene is stably expressed. The expression of the transgene must be continuously checked in order to ensure that the transgene

- is stable in the appropriate tissue and at the appropriate stage,
- effects the trait of interest,
- does not influence the performance of other agronomic traits.

Selection carried out over several generations not only confirms the stability of the transgene, but also allows the transgenic variety to be screened for possible mutations or gene silencing, which would prevent its being used in practice. The unfavourable effects of mutations, in the form of negative developmental or agronomic traits arising as the result of the transformation, can be corrected by backcrossing the variety to the original genotype in such a way that the transgene is stably expressed in the backcross generations.

If the transgenic lines are to be suitable for use as transgenic varieties, in addition to the stability of the transgene it is also important to test

- the agronomic traits of the variety in comparison with those of the original, non-transformed variety
- the adaptability of the variety under various agroecological conditions
- the environmental risks which may be encountered if it is commercially grown
- the safety and profitability of seed production, which will influence the competitiveness of the variety.

Agronomic analysis of a transgenic wheat variety

Little work has as yet been published on the changes in agronomic performance observed due to the transformation technology compared with the original, non-transformed genotype. In order to study this question, a joint

British-Australian-Hungarian experiment was set up to study the agronomic properties of a transgenic wheat variety, with special attention to the technological quality under various agroecological conditions (Rakszegi et al., 2001).

The transgenic spring wheat variety B73-6-1 has extra copies of the 1Dx5 HMW glutenin gene. When compared with the non-transformed variety in the field no difference was found between the transgenic and original varieties for yield performance, despite the fact that the transgenic variant had significantly smaller thousand kernel mass. It is well known that lower thousand kernel mass may occur for a number of reasons and may influence the protein and gluten contents.

The results indicated that, as a result of transformation with the HMW glutenin gene, there was a considerable change in several traits influencing technological quality (Table 2). In addition to the change in thousand kernel mass there was also an increase in protein content and in wet gluten content. The extra HMW glutenin subunit in the transgenic variety B73-6-1 led to greater grain hardness and hardness index, which had a positive influence on the farinograph water absorption.

Among the 10 g mixograph parameters used to determine functional properties, the dough formation time became longer, while the other characters were less favourable than those of the original, non-transformed variety. This negative change in spite of the greater protein content can be attributed to the fact that, due to the overexpression of the 1Dx5 subunit, the dough was overstrong and, due to the larger number of disulphide bridges, a satisfactory gluten skeleton did not evolve, upsetting the equilibrium between the extensibility and elasticity of the dough. In further experiments the flour of the transgenic wheat variety was mixed with that of a poorer quality wheat, thus improving the quality of the flour of the traditional variety.

Table 2

Yield performance and functional properties of the original wheat variety L88-6 and its transgenic variant B73-6-1

Yield performance and functional properties	L88-6	B73-6-1
Yield (kg/plant)	0.94	0.96
Thousand kernel weight (kg)	34.19	30.55
Test weight (kg)	78.58	77.58
Hardness index	14.43	35.14
Water absorption (ml)	50.35	51.00
Farinograph number	88.00	13.60
Chopin W*10 ⁻⁴ I	125.63	68.00
Protein (%)	11.95	12.60
Gluten (%)	28.25	28.75

In the course of the field experiments the potential environmental danger represented by the transgenic wheat was examined in the light of the Hungarian regulations, which are in line with the strictest international guidelines. On the basis of these studies it can be stated that no environmental damage whatsoever was experienced during the cultivation of the transgenic wheat.

The results of the field experiments also demonstrated that the incorporation of a single alien gene may have both a positive and a negative influence on the agronomic properties of the transformed plant. After the completion of the technological stage of the transformation it is therefore of great importance to test all the agronomic properties of the transgenic plant before it is introduced into cultivation.

Difficulties encountered during the application of the gene technology in plant breeding

Plant breeders cannot ignore public opinion when forming research concepts. This is particularly true of European plant breeders, since it is in this region that the greatest differences are found in consumer behaviour. As a consequence, European plant breeders are at a disadvantage compared to their colleagues in other regions in the application of gene technology methods. According to a survey carried out in 2000 (Arundel et al., 2000), European breeders are doing their best to catch up. Some 33% of the 99 European breeding companies which took part in the survey stated that they used gene technologies in addition to conventional breeding. Other companies also intend to introduce the technology by 2002, by which date this ratio should thus be 49%. A further 31% of the companies intend to use marker technology and gene sequencing as supplementary techniques, compared to 23% in 1999. All in all it can be said that four out of five European breeding companies include molecular breeding methods in their programmes in some form in addition to traditional breeding techniques.

One obstacle to the application of gene technologies in breeding is the great expense involved in the research and in the introduction of transgenic varieties. These costs are only returned at present for a few crops grown on large areas, so this limits the spread of these techniques to a wider range of agricultural crops. Due to the introduction of even stricter safety regulations, these costs are likely to rise even more in future. This will result in a further concentration of plant breeding, with the consequence that

- the genetic stocks employed in breeding will become more restricted
- the reduced genetic diversity caused by the possible germplasm exchange restrictions will be associated with an increase in the potential risk of genetic vulnerability.

Conclusions

The aim of molecular plant breeding is to use gene manipulation techniques to induce beneficial changes which cannot be achieved at all, or with far lower efficiency, by means of traditional breeding. By transforming cereals it is possible to increase the agronomic performance of the plant, improve the efficiency and stability of production, and broaden the end uses of the crop. Gene technology is just one phase in the development of a new plant variety. The development of a transgenic variety requires the isolation of a gene or gene section from the donor genome for the purpose of transformation, a homozygous plant or target genome developed by traditional breeding and suitable for transformation, an efficient transformation protocol and the development of a transgenic variety from fertile transformed plants.

Conventional breeding methods must be used to make the transgenic plant suitable for variety development. The transgenic variety, which must meet DUS requirements, must have satisfactory agronomic traits and a stable yield, its seed production must be profitable, and the changes induced by the transgene should give it an economic advantage over the original variety, making it of commercial value.

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MAPPING OF GENES REGULATING ABIOTIC STRESS TOLERANCE IN CEREALS

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The location of major QTLs or even genes controlling abiotic stress tolerance is now possible by the application of marker-mediated techniques. This is achieved by exploiting precise genetic stocks, such as doubled haploids (DHs), recombinant substitution lines (RSLs) and recombinant inbred lines (RILs), along with the comprehensive genetic maps now available through the application of molecular marker techniques. These strategies are illustrated here showing how QTLs/genes affecting vernalization response, cold tolerance, osmotic adjustment, osmolite accumulation (free amino acids, polyamines and carbohydrates), salt tolerance and cold-regulated protein accumulation have been identified and located. Also, an example of marker-assisted selection (MAS) for frost tolerance is presented. Major loci and QTLs affecting stress tolerance in *Triticeae* have been mapped on the group 5 chromosomes, where the highest concentration of abiotic stress-related QTLs (vernalization response, frost tolerance, salt tolerance and osmolite accumulation) was located. A conserved region with a major role in osmotic adjustment has been located on the group 7 chromosomes.

Key words: abiotic stress, QTLs, mapping, wheat, drought tolerance, frost tolerance

Introduction

Abiotic stress tolerance, including frost, drought and salinity tolerance, has long been regarded as a trait subject to complex multigenic control, and most variation seems to be mediated by QTLs. However, it is still possible that major differences in stress adaptation between species or cultivars may depend on allelic differences in a small number of genes, as suggested for cold acclimation in pea and *Solanum* (Liesenfeld et al., 1986; Stone et al., 1993) or for genes controlling responses to drought (Quarrie et al., 1995). However, because of the complexities of measuring stress responses at the phenotypic, physiological and biochemical levels, much less is presently known about the genetic control of abiotic stress responses than about other more highly heritable traits like flowering time (Snape et al., 2001) or disease resistance (Gupta et al., 1999). The location of genes controlling these complex traits is now possible by the application of marker-mediated techniques. Such modern methods of genetic analysis have led to the location of major genes and QTLs mediating the tolerance of abiotic stresses in cereals. This is achieved by exploiting precise genetic stocks, such as doubled haploids (DHs), recombinant substitution lines (RSLs) and recombinant inbred lines (RILs), along with the comprehensive genetic maps now available through the application of molecular marker techniques (Galiba et al., 1997a; Zhang et al., 1999; Snape et al., 2001; Cattivelli et al., 2002).

The basis of comparative genetics

History

'Comparative genetics' is the science that exploits the results of 'comparative mapping' – two terms that were unknown to plant geneticists 15 years ago (Devos and Gale, 1997). Although the discovery that genes in related species tend to be ordered collinearly on chromosomes is quite new, the concept of conservation has a long history in plant genetics. In the 1920s Vavilov already observed that 'similar variations' were to be found in different species. More recently, DNA studies have shown that genes of similar function in different species have remarkably conserved sequences. The discovery of collinearity by comparative mapping is, however, a function of the new molecular markers – in particular RFLPs – employed in plants for the first time in the mid-1980s. Gene collinearity was first reported in 1988 based on the convergence of the maps of the three genomes of hexaploid bread wheat (Chao et al., 1988) and the similar convergence between the maps of tomato and potato (Bonierbale et al., 1988).

Location of markers on chromosomes

It is often desirable to locate markers on chromosomes or even parts of a chromosome before genotyping a large set of individuals. To locate a locus on a chromosome or chromosome arm, one uses some type of cytogenetic stock. Bread wheat (*Triticum aestivum* L.) is an allohexaploid species ($2n = 2x = 42$) with the genome constitution AABBDD, formed through the hybridisation of *T. urartu* (AA) with a B genome diploid of unknown origin, and subsequent hybridisation, only about 8000 years ago, with the D genome diploid *T. tauschii*. Extensive studies showing the close genetic similarities between these genomes were begun by the late Prof. Ernie Sears, who was able to synthesize numerous aneuploid stocks in the wheat variety Chinese Spring (Sears, 1954). The development of aneuploid stocks led to the discovery that an extra dose of a particular chromosome could compensate for the absence of another, since the aneuploids are easier to maintain if the missing chromosome pair is compensated by the duplication of a homoeologous pair of chromosomes from the other genomes. This condition is called nullitetrasomy. This compensating ability of chromosomes of different ancestral origin resulted in the classification of the 21 wheat chromosomes into 7 homoeologous groups (Sears, 1958). Similar compensating experiments determined the homoeologous relationships between wheat chromosomes and those of other *Triticeae* species such as rye, barley and several *Aegilops* ssp. The ability of chromosomes to substitute for one another suggests that they carry similar genes.

Other useful cytogenetic stocks are the mono- or ditelosomes for individual chromosomes; in the 1AS ditelosome, for example, the 1A chromosome consists of two copies of the short arm, while the long arm is absent. These cytogenetic stocks are useful for localizing genes on a specific chromosome arm and for estimating their distance from the centromere. More recently, Endo and Gill (1996) developed deletion stocks that have homozygous deletions of different length for each chromosome arm.

Comparative genetics at the map level

The application of RFLP markers in plant genetics provided, for the first time, unlimited supplies of markers of both genic (cDNA probes) and random genomic (genomic DNA probes) origin to detect variation at the DNA level. The hybridization of cDNA clones to aneuploid lines of hexaploid bread wheat showed that most genes were triplicated on the A, B and D genomes (Devos and Gale, 1997). Generally, these probes cross-hybridized strongly under high stringency conditions to wheat relatives such as barley, oat and rye. The first publications that demonstrated the breadth of synteny among the cereals were those of Ahn and Tanksley (1993) showing the relationship between rice and maize, Kurata et al. (1994) showing that the wheat genome could be aligned with rice, and Moore et al. (1995) who showed that all maps could be combined in a single synthesis.

Mapping populations and strategies

In deriving a linkage map for particular species, one must first develop a mapping population that will produce segregation at the desired loci (Snape et al., 2001). Many types of populations are possible, and decisions regarding which types are used are based on the desired resolution and the ease or possibility of development. F_2 and backcross populations are adequate and easy to produce for self-pollinated species like wheat. These strategies are fast but produce limited amounts of seed of the mapped individuals. If multiple tests in multiple locations are necessary to measure the targeted character, it is necessary to develop immortal mapping populations such as recombinant inbred lines (RILs), recombinant substitution lines (RSLs), or doubled haploids (DHs). To produce RSLs a substitution line is crossed with the normal line. The hybrid is like the F_1 , but only for the substituted chromosome. The F_1 is crossed as a male with a cytogenetic stock of the same line lacking that chromosome (nullisomic). In the progeny the recombined chromosome will have no companion to pair (monosomic). The monosomic plant is self-pollinated and the recombined chromosome is recovered in a double dose (Law, 1966).

Bread wheat possesses a large genome (16 billion bp per haploid genome), which is about six times the size of the maize genome and 35 times that of rice. The three crops most probably originated from a common ancestor some 60 million years ago (Bennetzen and Freeling, 1993). Besides polyploidy in wheat, a key step in the evolution of these crops was the differential amplification of DNA, to a greater extent in wheat than in maize or rice. The genes in wheat may be present in uninterrupted clusters, individually interspersed by repetitive DNA blocks. Gill and Gill (1994) proposed a mapping strategy to target gene-rich regions of the wheat genome. Briefly, single-break deletion lines are used to divide each wheat chromosome into small regions marked by chromosome bands, protein and DNA markers. The resultant

physical maps are then compared with the corresponding genetic linkage maps to analyse the physical distribution of recombination and the order of markers within each chromosome region. The physical map is compared with a genetic linkage map by drawing lines to join the common markers. The resultant composite map is called a cytogenetic ladder map (CLM). This method was used successfully to identify and preferentially map gene-rich regions in homoeologous group 5 chromosomes of wheat (Gill et al., 1996).

Mapping genes for vernalization requirement and stress responses to the 5th homoeologous chromosome group of wheat

Mapping of genes for flowering time and frost resistance

Major genes controlling sensitivity to vernalization, the *Vrn* genes, determine the control of the spring wheat/winter wheat growth habit difference. Winter types require vernalization to initiate flowering and spring types do not. The lack of vernalization requirement is generally dominant. Five loci are known to control spring/winter differences and the chromosomal locations of four of them have been established using chromosome substitution line analysis, namely *Vrn-A1* (on chromosome 5A), *Vrn-D1* (on chromosome 5D), *Vrn-B1* (on chromosome 5B) and *Vrn-B4* (on chromosome 7B) (Law et al., 1976; 1991). Studies have shown that alleles at the *Vrn-A1* locus appear to be predominant in reducing vernalization requirement (Snape et al., 1985).

In addition, although studies have shown that the genetic control of frost resistance is complex and can be regarded as a polygenic trait (Sutka, 1981), major genes have been mapped on chromosomes 5A and 5D using molecular markers (Galiba et al., 1995; Snape et al., 1998). The major question is, therefore, whether vernalization response and cold tolerance are pleiotropic effects of the *Vrn* genes or determined by separate loci. To answer this question requires precise mapping of the loci involved in both traits, using a marker-mediated approach.

To map genes affecting flowering time and frost tolerance on chromosome 5A, a population of single chromosome recombinant lines, derived from the cross between the single chromosome substitution lines Chinese Spring (*Triticum spelta* 5A), which is vernalization insensitive (spring habit) + frost sensitive, and Chinese Spring (Cheyenne 5A) which is vernalization sensitive (winter habit) + frost tolerant, was developed. The flowering time of each line was characterised in growth chamber under a 16/8 h day/night regime at temperatures of 22 and 15°C, respectively, without prior vernalization. Frost tolerance was assessed using low temperature treatments varying from -10 to -15°C following hardening. Discontinuous variation for flowering time and frost responses was observed when the phenotypes were assessed under these conditions. RFLP mapping techniques were applied to the lines to develop and anchor the genetic map, and this showed that *Vrn-A1* and *Fr1*, the locus for cold

tolerance, were separate, but closely linked loci, on the long arm of chromosome 5A. Both loci were closely linked to the RFLP markers *Xpsr2021(ABA2)*, *Xwg644* and *Xpsr426*, distally on the long arm, but proximal to the 5A/4A translocation.

The analysis of chromosome 5A has been extended to examine if there are homoeologous loci for vernalization and stress responses, particularly frost tolerance, on chromosome 5D (Snape et al., 1997). A recombinant substitution line population was developed from the cross between Chinese Spring, which is vernalization insensitive, and the single chromosome substitution line Chinese Spring (Cheyenne 5D), which is vernalization sensitive. Since previous work has shown that chromosome 5D has few RFLPs, the strategy used for mapping was to use the RFLP probes previously located on the homoeologous group 5 chromosomes to search for polymorphisms to provide 'anchor markers' for the map and then use Amplified Fragment Length Polymorphism (AFLP) and microsatellite markers (simple sequenced repeats, SSRs) to provide greater resolution. Each line was characterised phenotypically for flowering time and frost tolerance as described above. *Vrn-D1* was located distally on the long arm of 5D, closely linked to the SSR markers *Xgwm212* and *Xgwm292*. QTL analysis for cold response revealed that the locus of the *Fr2* gene is a linked locus and not a pleiotropic effect of *Vrn-D1*, since the maximum likelihood position was about 6 cM proximal to *Vrn-D1*.

Comparative mapping

The individual mapping data for the 5A and 5D chromosomes show that the *Vrn-A1* and *Vrn-D1* loci are probably homoeoallelic. To prove this further, the maps were aligned using cross hybridizing probes and 'bridging' maps of 5A and 5D developed on a doubled haploid population from the cross of Chinese Spring \times SQ1 (Quarrie et al., 1995). This revealed that *Vrn-A1* and *Vrn-D1* are, in all probability, homoeoallelic, like *Fr1* and *Fr2*. These loci are thus probably derived from the common ancestor of the A and D genomes and pre-date the separation of bread wheat.

In barley, winter habit depends on the presence of the dominant allele at locus *Vrn-H2* (formerly *Sh*) and of the recessive alleles at the loci *Vrn-H1* (*Sh2*) and *Vrn-H3* (*Sh3*). All the other allele combinations among these three genes are found in spring or facultative genotypes (Cattivelli et al., 1994). The loci *Vrn-H2*, *Vrn-H1* and *Vrn-H3* are located on the long arms of chromosomes 4H, 5H and 1H, respectively. A comparison of a common set of RFLP markers suggested that the orthologous position on the long arm of wheat chromosomes 5A, 5B and 5D, carrying the *Vrn-A1*, *Vrn-B1* and *Vrn-D1* genes, corresponds to the *Vrn-H1* locus of barley (Laurie et al., 1995; McIntosh et al., 1998) and to the *Vrn-R1* (formerly *Sp1*) vernalization response locus of rye (Plaschke et al., 1993).

Using rice probes supplied by the Japanese Rice Genome Program and a mapping population developed between the *indica* variety IR20 and the *japonica* variety 63-83 it was shown that a region homoeologous to the *Triticeae Vrn* region exists on rice chromosome 3. This finding was confirmed using deletion lines for chromosome 5A, developed in the variety Chinese Spring (Gill et al., 1996). Probes from rice chromosome 3 and probes co-segregating with *Vrn-A1* were all mapped between deletion breakpoints 0.68 and 0.78. Intriguingly, this region in rice contains a flowering time QTL, *FLTQ1*, mapped by Sarma et al. (1998) in the marker interval *psr145* to *R3226*. Moreover, other markers (*R2404*, *R2311*) associated with *Hd-6*, another flowering QTL which was mapped to the long arm of rice chromosome 3 by Yamamoto et al. (1996), have been mapped to the same chromosomal region of the deletion lines flanked by breakpoints 0.68 and 0.78. However, the present data are not sufficient to determine whether these QTLs, *FTLQ1* and *Hd-6*, are the same or different loci.

Marker-assisted selection

Storlie et al. (1998) were interested in determining the utility of the *Vrn-A1-Fr1* interval on the long arm of chromosome 5A for improving cold hardiness in a winter wheat breeding programme. The interval contains genes influencing vernalization requirements and cold hardiness levels. This was accomplished by examining the freezing tolerance of near-isogenic lines (NILs) carrying the *Vrn-A1-Fr1* intervals from different varieties. In particular, NILs were derived from five back-crosses between Marfed, a freezing-sensitive (LT_{50} : $-8.2^{\circ}C$) spring wheat that was used as recurrent parent, and two winter wheat donor parents that differed in freezing tolerance, Suweon 185 (LT_{50} : $-13.6^{\circ}C$) and Chugoku 81 (LT_{50} : $-12.7^{\circ}C$). An analysis of the progeny indicated that those carrying the winter *vrn-A1-fr1* locus were about $4^{\circ}C$ more freezing tolerant than those carrying the spring *Vrn-A1-Fr1* locus. (Freezing sensitivity (*Fr*) is dominant over freezing tolerance (*fr*).) Similarly, progeny carrying the *vrn-A1-fr1* locus from Suweon 185 were about $0.5^{\circ}C$ more freezing tolerant than those carrying the *vrn-A1-fr1* locus from Chugoku 81. The *Vrn-A1-Fr1* interval accounted for 70 to 90% of the difference in the freezing tolerance of the NILs, substantiating the importance of this locus in cold acclimation. In addition, the results indicate that differences in freezing tolerance between winter wheat cultivars can, in at least some cases, result from differences at this locus.

Mapping of genes contributing to general stress tolerance

Genes affecting osmotic adjustment

Osmoregulation is a process which partly or fully maintains turgor pressure or cell volume by increasing the amount of solute inside the cell when water potentials decline outside the cell. Osmotic adjustment refers to the lowering of osmotic potential due to the net accumulation of solutes in response

to water deficit (Zhang et al., 1999). In wheat, large differences in response exist between genotypes. The osmoregulation differences are conditioned by alternative alleles at a single locus (*OrOr/oror*), with high response being recessive (Morgan, 1991). The gene appears to primarily condition a difference in potassium accumulation, with amino acid accumulation as a possible secondary and dependent response (Morgan, 1992). Analysis of a single chromosome substitution series of Chinese Spring/Red Egyptian indicated a location on chromosome 7A (Morgan, 1991). The position of the *Or* gene was specified using an NIL (near isogenic line) mapping population originating from a cross between the cultivar Songlen (high response) and the breeding line Condor (low response). The *Or* locus was mapped on the short arm of chromosome 7 approximately 13 cM towards the centromere from the RFLP locus *Xpsr119* (Morgan and Tan, 1996).

Research was conducted to identify and map quantitative trait loci (QTL) associated with the dehydration tolerance and osmotic adjustment of rice. The osmotic adjustment capacity and lethal osmotic potential were determined for 52 RILs grown in a controlled environment under slowly developing stress conditions. The lines were derived from a cross between an *indica* cultivar with lowland adaptation, Co39, and a traditional upland *japonica* cultivar, Moroberekan. One-way analysis of variance and interval mapping were conducted to detect QTLs controlling osmoregulation. A major QTL explaining one-third of the phenotypic variation for osmotic adjustment (OA) was identified at the RG1 region of chromosome 8 (Lilley et al., 1996). It is worth pointing out that the region of rice chromosome 8 containing the OA QTL is homoeologous with a segment of wheat chromosome 7 (Ahn et al., 1993), where the single *Or* locus described above was identified. A comparative map was drawn showing the conserved region between rice and wheat (Zhang et al., 1999). The QTLs identified were compared to root traits and leaf rolling scores measured on the same line in this rice experiment. Osmotic adjustment and dehydration tolerance were negatively associated with root morphological characters involved in drought avoidance. High osmotic adjustment and dehydration tolerance were associated with the Co39 alleles and extensive root systems were associated with the Moroberekan alleles. To combine high osmotic adjustment with extensive root systems, the linkage between these traits (closely linked to the same *RG1* region of chromosome 8) will have to be broken.

Genes affecting osmolite accumulation under stress conditions

Galiba et al. (1992) reported that genes controlling osmotic adjustment in wheat tissue culture are primarily located on chromosomes 5A and 5D. This observation was based on stress-induced free amino acid accumulation profiles in a series of Chinese Spring (Cappelle Desprez) substitution lines. It appears that the genes identified by Galiba et al. (1992) regulate free amino acid as well as polyamine accumulation (Galiba et al., 1993), and thus differ from the gene

found by Morgan (1991). Recent studies on the genetic regulation of carbohydrate content in the course of cold hardening supported this assumption. The frost resistance and endogenous carbohydrate contents of the plants were measured every 8 days in the course of cold treatment (Vágújfalvi et al., 1999). There was a continuous rise in the total water-soluble carbohydrate (WSC) content and in the fructan content as the hardening period proceeded. The increase in concentration was greater in the frost-resistant variety Cheyenne and in the substitution lines Chinese Spring (Cheyenne 5A), Chinese Spring (Cheyenne 5D) and Chinese Spring (Cheyenne 7A) than in the sensitive genotypes Chinese Spring and Chinese Spring (*Triticum spelta* 5A). A significant positive correlation was found between frost resistance, WSC and fructan content after 19 days of cold treatment. A significant correlation was exhibited between the fructose and sucrose contents and frost resistance after 43 days of cold treatment. In the chromosome substitution lines the accumulation of carbohydrates began after 11 days of cold hardening and reached a maximum after 35–43 days.

The sucrose and fructan contents in recombinant lines arising from a cross between the substitution lines Chinese Spring (Cheyenne 5A) and Chinese Spring (*Triticum spelta* 5A) were determined after cold hardening (Galiba et al., 1997b). The *Fr1* (frost sensitivity) and *Vrn-A1* (spring habit) alleles originating from *Triticum spelta* did not increase the sucrose and fructan contents, while their concentrations increased significantly in recombinant lines carrying the alleles *vrn-a1* (winter habit) and *fr1* (frost resistance). In the 7-3 line, carrying the *vrn-a1* and *Fr1* genes (where recombination took place between the *Fr1* and *Vrn-A1* genes), a large accumulation of sugar was observed, indicating that the allele influencing carbohydrate accumulation was closely linked to the *Vrn-A1* gene. This confirms that these genes may contribute to general stress tolerance through mechanisms which act at the cellular level.

Genes affecting salt tolerance

Salt tolerance in cereals is known to be associated with the control of shoot Na^+ content; tolerant genotypes have more efficient systems to exclude sodium from their shoots. A single locus (*Kna1*) localised on chromosome 4D of wheat was shown to control K^+/Na^+ discrimination. Indeed, in a saline environment, bread wheat (genomes AABBDD) accumulates less Na^+ and more K^+ than durum wheat (genomes AABB) (Dubcovsky et al., 1996). The analysis of wheat cytogenic stocks has demonstrated that the homoeologous group 5 chromosomes carry loci involved in the response to salt stress under hydroponic conditions (Koeberner et al., 1996). Chromosome 5E, derived from the wild species *Thinopyrum bessarabicum* or *Lophopyrum* (*Thinopyrum*) *elongatum*, was shown to increase wheat salt tolerance (Kasai et al., 1998). QTLs controlling salt tolerance at germination (chromosomes 1H, 4H, 5H and 6H) and at the seedling stage (chromosomes 1H, 2H, 5H and 6H) have been mapped in

cultivated barley (Mano and Takeda, 1997). Notably, the two QTL sets do not overlap, a finding in agreement with previous evidence suggesting that different genetic mechanisms control salt tolerance during germination and plant growth (Mano and Takeda, 1997).

Mapping of genes affecting cold-regulated proteins

Messenger RNAs corresponding to the cold-regulated gene *cor14b* (formerly *pt59*) are accumulated in barley leaves when plants are exposed to low temperature. The expression of *cor14b* is strictly regulated by cold (Cattivelli and Bartels, 1990), although it is enhanced by light-dependent factor(s) (Crosatti et al., 1999). Further studies have demonstrated that the *cor14b* gene encodes for the COR14b protein, which is cold regulated and imported into the chloroplast (Crosatti et al., 1996). Much experimental evidence suggests a relationship between the accumulation of the COR14 proteins and frost resistance. In particular, it has been demonstrated in barley that the threshold induction temperature for COR14 proteins is lower in frost-sensitive than in frost-tolerant cultivars (Crosatti et al., 1996) and, when evaluated under field conditions, winter barleys accumulated more COR14b than spring ones (Giorni et al., 1999). A gene homologous to *cor14b* is expressed in response to low temperature in other monocots including wheat (Cattivelli and Bartels, 1990).

Vágújfalvi et al. (2000) reported on the genetic regulation of the wheat gene homologous to the barley *cor14b*. Among the wheat plants raised under control (18/13°C day/night) conditions the accumulation of *cor14* mRNA was observed in the leaves of frost-resistant genotypes, but not in those of frost-sensitive varieties and lines (Vágújfalvi et al., 2000). At higher temperatures (25/18°C) there was no detectable quantity of *cor14* mRNA in any of the genotypes. At low (2°C) temperature all the varieties accumulated mRNA. As experienced previously in barley, gene expression was temperature- and tolerance-dependent, though the temperature threshold at which the gene became expressed was higher in wheat than in barley. This result was confirmed by the Western blot analysis of the COR 14b protein. This was followed by the mapping of the gene regulating the expression of the *cor14b* gene. The results of Western and Northern analyses showed that the 5A chromosome carries genes responsible for the sensing of the threshold temperature. In the Chinese Spring genetic background at 2°C the Cheyenne 5A chromosome increased the quantity of *cor14b* mRNA. At 18/13°C the COR 14b protein was only present in demonstrable quantities in the Cheyenne variety and in the Chinese Spring (Cheyenne 5A) line, but not in the Chinese Spring parent or in the Chinese Spring (*Triticum spelta* 5A) line.

The analysis of single chromosome recombinant lines derived from the cross between Chinese Spring (*Triticum spelta* 5A) and Chinese Spring (Cheyenne 5A) identified two loci with additive effect which are involved in the genetic control of *cor14b* mRNAs accumulation. The first locus was positioned

tightly linked with marker *psr911*, while the second one was located between marker *Xpsr2021* and the frost resistance gene *Fr1*. It was known that the structural gene was located on chromosome 2 in barley, but its position on the chromosome was unknown (Crosatti et al., 1996). The RFLP mapping of the structural gene was carried out using a *cor14b* gene cDNA probe on a *Triticum monococcum* mapping population. The locus of the *cor14b* allele examined was mapped on the long arm of the 2A^m chromosome.

We can only speculate on the role of the COR 14 protein. It probably helps to prepare the plants for cold hardening. Its accumulation in both wheat and barley was associated with a well-defined threshold temperature, so it is considered to be a useful biochemical marker for the selection of frost-resistant and frost-sensitive genotypes.

Conclusions

Cattivelli et al. (2002) have constructed a genomic map of major loci and QTLs affecting abiotic stress tolerance in *Triticeae*. According to this map chromosome group 5 has the highest concentration of QTLs and major loci controlling plant adaptation to the environment, particularly those controlling heading date, frost, salt and, to a lesser extent, drought tolerance. A region with a crucial role in drought tolerance (osmotic adjustment) is located on chromosome group 7. Although the molecular responses to cold and drought stresses share a common set of genes (Cattivelli et al., 2002), the loci describing the genetic bases of cold and drought tolerance are different. Nevertheless, multiple stress QTLs and linked markers have also been detected, suggesting the existence of common mechanisms for different stresses, or clusters of genes controlling different stress tolerance processes. The best example of this is the 5A chromosome of wheat.

Dubcovsky et al. (1995) mapped the dehydrin and *Esi* genes involved in osmoregulation and the loci of heat shock proteins to the region of the *Vrn* gene locus on the long arm of the fifth chromosome of *T. monococcum* (5A^mL). In addition, as described above, genes regulating abscisic acid and carbohydrate content and the accumulation of COR14b protein were also localised around the *Vrn-A1-Fr1* interval. These results indicate that the 5A chromosome carries an "adaptation gene complex" in the region of the *Vrn-A1* and *Fr1* genes. The development of this gene family regulating adaptation may have represented an evolutionary advantage. Recombination frequency is low for genes located close to each other, so the genes are inherited together and form a relatively large functional unit. As the result of continual selection pressure due to the winter climate, the grouping of the genes ensured a selective advantage for progeny generations.

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QTL ANALYSIS OF WHEAT QUALITY TRAITS

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This paper aims to give an overview on the different aspects of QTL analysis of quality traits of wheat through the brief introduction of molecular genetics, cereal chemistry and the statistical methods developed and applied recently in this area. Some examples are also provided, based on the author's research activity carried out in the National Wheat Molecular Marker Program (NWMMP) established in Australia in 1996.

Key words: QTL analysis, restriction fragment length polymorphism (RFLP), microsatellite DNA or simple sequence repeat (SSR) polymorphism, amplified fragment length polymorphism (AFLP), simple interval mapping (SIM), composite interval mapping (CIM), multiple interval mapping (MIM), small-scale dough testing, extensibility

Introduction

The success of wheat breeding has largely come from the application of new technologies to breeding and selection. Biotechnology offers new means for improving wheat, through genetic engineering and through the development and application of molecular markers. Molecular marker technology offers a wide range of novel approaches to improving the efficiency of selection strategies. These techniques are based around the detection of sequence variation between varieties or accessions of wheat. Where the sequence variation is located in a region of the genome closely linked to a trait of interest, the polymorphism can be used to predict the presence or absence of the trait (Langridge et al., 2001). The strength of the prediction will depend upon two factors: the closeness of the genetic linkage between the sequence variation and the target locus and the precise, well-defined measurement of the trait of interest. In the case of most of the traits related to wheat flour quality, this second requirement has become the critical issue to be able to utilize the potential advantages that biotechnology could offer in wheat breeding. Therefore, parallel with the intensive research effort in molecular biology, there is a concentrated effort in cereal science to provide better measurements for different aspects of quality attributes such as dough strength, extensibility or water absorption (Bekes et al., 2002; Gras et al., 2001).

This paper aims to give an overview on the different aspects of QTL analysis of quality traits of wheat through the brief introduction of molecular genetics, cereal chemistry and the statistical methods developed and applied recently in this area. Some examples are also provided, based on the author's research activity carried out in the National Wheat Molecular Marker Program (NWMMP) established in Australia in 1996 (Appels et al., 2001).

Mapping and analysis of quantitative trait loci in experimental populations

Traits that show a continuous distribution between two extremes are regarded as quantitative traits. This distribution pattern can arise for two major reasons: the trait is controlled by several loci each of small effect and different combinations of alleles at these loci can give different phenotypes, and/or the trait is strongly influenced by the environment so that a combination of genetic and environmental factors influences the phenotype. The identification of markers linked to loci controlling quantitative traits (QTLs) is substantially more complex than for simple traits. To date, most mapping of QTLs has depended upon the construction of complete linkage maps of populations segregating for the trait of interest. This is in contrast to simply inherited traits where bulked segregant analysis (BSA) is a rapid and effective means for finding linked markers (see below). In addition, where the quantitative trait is strongly affected by the environment, testing over multiple environments is usually required since this will allow a measure of the heritability of the trait and may permit correction of the data to allow for the environmental component. For the analysis of data from field trials, spatial analysis of the trial data can substantially increase the heritability of the trait and the ease of identifying controlling QTLs. Although QTL analysis represents a substantially more complex target for marker identification than simply inherited traits, the benefits to selection programmes of having markers associated with QTLs are far greater. These are usually traits that are difficult to select for in a conventional breeding programme but are frequently prime targets for wheat improvement. This applies for resistance to several major diseases, tolerance to a variety of abiotic stresses, and many major components of processing quality.

Mapping and tagging quality traits

Grain and flour quality traits are key targets for the development and application of molecular markers in wheat for several reasons. Many of the quality tests that are undertaken in breeding programmes require considerable amounts of grain, so the tests can only be performed in late generations of the programme. The quality of grain for various end products can be, and often is, affected by seasonal factors such as drought, frosting during grain development, or pre-harvest rainfall. This can mean, in particular years, that a considerable proportion of the breeding programme will not have particular quality tests performed, or the data obtained are unreliable. The difficulty of collecting quality trait information on populations and the low heritability of many quality components in wheat have meant slower progress in mapping and using markers for wheat quality traits than for the more easily defined malting quality traits in barley.

Despite these difficulties, significant progress has been achieved for wheat quality trait mapping and tagging. For bread dough properties, the effects of various alleles of the storage protein loci are well known. For example, the high and low molecular weight glutenin subunits (HMWGS and LMWGS, respectively) and gliadins are classified by protein analysis via SDS-PAGE and variations in many of these wheat storage proteins are known to be related to dough properties of bread wheat (Payne et al., 1979; Bekes et al., 2001; Cornish et al., 2001). As a consequence, many breeding programmes screen for these traits by SDS-PAGE analysis of the grain proteins in preference to the use of direct quality measurements. Many of the genes encoding these proteins are now cloned and sequenced, and PCR-based DNA tests for a number of these loci, or for particular alleles, are available for use in bread and durum wheat (e.g. D'Ovidio and Anderson, 1994; Ahmad, 2000). Such tests represent an alternative screening method as breeding programmes begin to use other DNA-based markers, since the DNA will be available.

However, it is also well known that not more than 70–75% in the variation in different dough quality parameters such as maximum resistance (RMAX) or extensibility can be explained by the variation in the storage protein composition of the grain. Based on the information collected in cereal science in the last 50 years, differences in dough properties and baking quality are largely determined by the superimposed effects of protein content, glutenin-to-gliadin ratio and the size distribution of the polymeric glutenin. The size distribution of the polymeric glutenin largely depends on the HMWGS to LMWGS ratio, the allelic composition of HMWGS and LMWGS and the relative amounts of the different glutenin subunits. There is an expectation that the use of molecular technologies to investigate the relationships between the chemical composition and functional properties of wheat will discover not only those genes which determine the structural elements responsible for wheat gluten quality, but also those which are responsible for their relative amounts and macromolecular organisation.

Advances in the genetic mapping of wheat, the molecular interpretation of flour processing traits and large-scale sequencing of genes expressed in endosperm tissue are currently converging to define the genes that under-pin key quality traits (Appels et al., 2000). To achieve this, accurate definitions and measurements of phenotypes associated with the mixing of dough, such as dough extensibility, are essential. The development of small-scale dough testing equipment, and the associated automated interpretation of the resulting mixing curves has provided better reproducibility and removed operator bias, resulting in more objective assessment of the experimental variables. This has facilitated a wide range of research activities and several applications in breeding programmes where either only limited amounts of test material have been available or the more objective, precise assessment of data offered extra benefits. In breeding, the use of a smaller sample size provides an opportunity to grow and screen many more new lines than was previously possible. This in turn

minimizes the cost and the time of the breeding process, and maximizes the chance of breeding highly successful new lines (Gras and O'Brien, 1992). Small-scale dough testing has also been particularly useful in formulating relationships between genetics and quality, and chemical composition and functional properties (Bekes and Gras, 1999). Detailed studies carried out on the Mixograph (a pin mixer) have furthermore established that its mixing action develops the dough through successive elongation-rupture-relax cycles of the flour/water mixture (Anderssen et al., 1998; 2001). These studies have provided a theoretical basis for applying multiple regression analysis to develop predictive relationships between extensibility and the mixing properties of dough, resulting in the development of a new measure for extensibility, M-extensibility.

QTL mapping: theory and practice

Most wheat quality traits appear as quantitative traits, whereby several individual genes are involved and these may then interact with the environment to produce the phenotype. As a result, the trait values are continuously distributed, with each individual gene explaining only part of the observed trait variation (Tanksley, 1993). The genes conferring quantitative traits are referred to as quantitative trait loci, or QTLs. In order to breed high quality wheat varieties, it is essential in traditional wheat breeding programmes to have multi-environment field trials to sample genotype \times environment ($G \times E$) interactions. Significant quantities of grain (e.g. several kilogrammes) are usually required for subsequent quality testing. Such procedures are both time-consuming and expensive. Rather than direct analysis, the isolation of molecular markers associated with quality attributes will facilitate marker-assisted selection (MAS). This enables the selection procedure to be facilitated in the laboratory based on the marker genotypes and will potentially save a great amount of time and significantly reduce costs in breeding wheat varieties with the desirable quality attributes. The rationale behind MAS is that most DNA markers and some protein markers are phenotypically neutral (and therefore not subject to environmental effects), highly heritable and can be quickly and easily scored at the seed or seedling stage. For the purposes of single seed testing, many markers can be tested against DNA from as little as one half of one grain, leaving the proximal half of the grain for embryo rescue. Thus wide-scale field trials and expensive quality data acquisition can be significantly reduced, making the breeding procedure faster and more efficient.

Molecular markers

There are three DNA-based molecular marker systems widely used in wheat genetic mapping studies:

1. Restriction fragment length polymorphism (RFLP, Grodzicker et al., 1974): This molecular marker system is based on the differential hybridisation of

labelled cloned DNA to DNA fragments in a sample of restriction enzyme (RE) digested DNAs. The marker alleles are represented by a characteristic banding pattern for a specific probe/restriction enzyme combination.

2. Microsatellite DNA or simple sequence repeat (SSR) polymorphism (Tautz and Renz, 1984; Tautz et al., 1986; Weber and May, 1989): SSRs comprise sequences 1 to 6 nucleotides in length, tandemly repeated many times. Such sequences are hypervariable with respect to repeat length due to slippage during DNA replication. These markers are scored using a pair of polymerase chain reaction (PCR) primers that are complementary to the flanking region of SSRs to detect repeat length variation.

3. Amplified fragment length polymorphism (AFLP, Vos et al., 1995): This class of molecular marker is generated by a combination of restriction digestion and PCR amplification. Polymorphisms effecting RE recognition sequences or length variation between RE sites are scored.

Genetic populations

These are essential for the study of quantitative trait loci. F_2 , backcross (BC_1), recombinant inbred (RI), and doubled haploid (DH) populations are the four primary types of mapping population used in wheat.

F_2 populations are developed by selfing F_1 individuals from a cross between two parents lines showing significant variation for the trait of interest. Backcross populations (BC_1) are developed by crossing the F_1 with one of the two parents. Neither F_2 nor BC_1 populations are eternal, as they can only be used once. F_2 populations are not suitable for use with dominant markers, such as AFLPs. Two populations, each developed by backcrossing to one of the two parents, are necessary for scoring dominant markers with BC_1 populations (Crouzillat et al., 1996).

Recombinant inbred (RI) populations overcome the problems associated with the use of F_2 and BC_1 populations. They are developed by single-seed descent from individual F_2 plants. Single-seed descent is repeated for several generations to achieve near homozygosity for each RI line. It is possible for the RI populations to be replicated and therefore the RI population is suitable for a wide range of genetic studies. Moreover, genetic linkage maps derived from RI lines have higher resolution than those derived from F_2 or BC_1 populations (Burr et al., 1988). This is due to the increased number of meiosis events that occur during the inbreeding process.

Doubled haploid populations have similar genetic advantages to those of RI populations in that they are pure homozygotes and can be evaluated in replicated trials over years and sites. There are several ways to develop DH populations in wheat, the most popular being the crossing of wheat and maize (Kammholz et al., 2001). Dominant marker such as AFLPs can be successfully used, as every locus is homozygous within the DH population. Production of a DH population requires less than one year, while six or more generations of inbreeding are required to develop RI populations. This time-consuming process

is the major drawback of RI population production. In addition, DH populations have a higher degree of homozygosity than RI populations. However, as only one meiosis has occurred during their production, DH populations do not yield an improvement in map resolution relative to the use of F_2 or BC_1 populations.

Genetic linkage map construction

A genetic linkage map can help to estimate the relationships between markers and QTLs. A linkage map is normally constructed using one of three map functions – Morgan, Haldane or Kosambi (Liu, 1998). The Morgan map function uses the estimated recombination frequency as map distance (Morgan, 1928). However, due to multiple recombination events, the recombination frequencies are not additive, which means that the recombination frequency between two loci flanking a region is not the simple sum of the recombination fractions of the adjacent loci within the region. The Haldane and Kosambi map functions are designed to overcome this problem (Haldane, 1919; Kosambi, 1944). For the Haldane map function, the genetic distance (m) is estimated by $m = -0.5 \ln(1-2r)$, and for the Kosambi map function by $m = 0.25 \ln[(1+2r)/(1-2r)]$, where r corresponds to the recombination frequency between a pair of loci, and m is the distance between them in Morgans. Theoretically, the Morgan map function assumes complete interference (i.e. no double crossovers occur). In contrast, the Haldane map function assumes an absence of interference (observed double crossovers are equal to expected double crossovers), while the Kosambi map function assumes that the crossover interference depends on the size of a genome segment (i.e. the interference is absent when a segment is sufficiently large, and increases as the segments decrease in size). The three map functions are not universally applicable, as for example, Haldane and Kosambi map functions do not estimate physical distance in most cases (Liu, 1998).

Summary of QTL mapping methods

Statistical methods for finding the associations between quantitative traits and molecular markers have developed rapidly in recent years. An extensive review of this subject has been made by Zeng (1994).

1. Single marker analysis

Single marker analysis does not require a genetic linkage map, as it simply compares phenotype values of different marker types and identifies the associations between markers and traits. The significance of the association can be tested using any of the following tests (Liu, 1998): simple t-test; analysis of variance; linear regression; likelihood ratio test or maximum likelihood estimation. The first widely adopted QTL detecting method used analysis of variance (ANOVA, Soller et al., 1976). If the difference in trait value between different marker genotypes is found to be significant, it is taken to indicate that this marker may be linked to one or more QTLs. This single marker analysis has

two major limitations. It fails to indicate whether a marker is associated with one or more QTLs, and it does not give the likely position of a QTL on the chromosomes.

2. Simple interval mapping (SIM)

This was proposed (Lander and Botstein, 1989; Haley and Knott, 1992) to address the limitations of single marker analysis. With this method, a genetic linkage map must be first constructed. QTL analysis is conducted at and between mapped marker loci. This allows one to plot the amount of evidence for a QTL along the genome. There are several advantages of the SIM method over single marker analysis. First of all, the probable position of the QTL can be inferred by the significant interval and, secondly, the method requires a smaller population size than the single marker analysis approach. Simple interval mapping (SIM) can be implemented either using the maximum likelihood method (Lander and Botstein, 1989) or the linear regression procedure (Haley and Knott, 1992). The latter is actually a simplification of the maximum likelihood method. The estimates of QTL effects and positions are almost identical with these two methods (Tinker and Mather, 1995; Xu, 1995; 1998).

3. Composite interval mapping (CIM)

Whilst the SIM procedure works well when there is only one QTL located on the test chromosome, problems arise with the presence of multiple QTLs on a chromosome. The result of the SIM will be affected by other QTLs on the same linkage group, so their estimated positions and effects of QTLs are likely to be biased (Zeng, 1994). According to Zeng (1994), the mathematical basis of this model is not efficient, as it only uses two markers at a time for analysis, and the information from other markers is not utilised. A solution for overcoming the limitations of SIM was proposed by Zeng (1993, 1994) and Jansen (1993). This is actually a combination of simple interval mapping and a multiple regression procedure. With this method, simple interval mapping (SIM) is performed in the usual way, except that the variance from other QTLs is accounted for by including partial regression coefficients from markers in other regions of the genome. Partial regressions of markers from other regions of the genome (referred to as cofactors or background markers) are also included in every test, and the affects from other QTLs on the testing position can be reduced or absorbed by other markers in the model (Zeng, 1994).

4. Multiple interval mapping (MIM)

This is an extension of CIM, proposed by Kao et al. (1999). The CIM method uses one QTL and many markers. While CIM is essentially a one-QTL model, MIM is a multiple-QTL model. It uses multiple marker intervals simultaneously to fit multiple putative QTLs directly in the model for mapping QTLs. With this approach, the precision and power of QTL mapping could be improved. Also, epistasis (see below) between QTLs, genotypic values of individuals, and heritabilities of quantitative traits can be readily estimated and

analysed. The limitation of this approach is that it imposes a heavy computational burden and requires much greater computation resources and time than one-QTL approaches (SIM and CIM).

5. Estimation of epistatic effects

Epistatic effects are due to interactions between different loci. These include conditional and coadaptive epistasis (Chase et al., 1997). Conditional epistasis suggests that the primary additive effects of a QTL are conditional upon the presence of a particular allele from another locus, which either magnifies or reduces the primary QTL's effect. In contrast, coadaptive epistasis indicates that one locus has no genetic effect when it acts independently; however, with the presence of another specific allele, this locus has genetic effects. Detecting epistatic interactions is practically important, as it both provides information of behaviours of mapped QTLs in different genetic backgrounds (conditional epistasis), making marker-assisted selection more accurate, and provides an additional source of variation (coadaptive epistasis) to those from additive and dominant genetic effects. Methods for estimating epistasis have been proposed by Chase et al. (1997), Wang et al. (1999) and Kao et al. (1999).

Some examples

All examples presented are based on a doubled haploid population (Cranbrook \times Halberd, Kammholz et al., 2001) consisting of 161 lines. Quality traits were measured based on three field trials, including the varieties Stow (QLD, 1997), Roma (QLD, 1997) and Roseworthy (SA, 1996).

Example 1: Study of glutenin allelic composition effects on dough rheological properties

Mapmanager/QT (Manly and Olson, 1999) was used to calculate the additive genetic effects, while Epistat (Chase et al., 1997) was used to estimate the epistasis between different loci. The results indicated that the *GluB3* and *GluD3* (low molecular weight glutenin subunit) loci have highly significant additive effects on dough extensibility, while *GluA3* has no significant effect. The results were consistent among the three field trials. In addition, significant epistatic interactions were detected between *GluB3* and *GluD3*. Tables 1 and 2 indicate that the genetic effects of *GluB3* (allele *d* over *c*) can only be detected in the *GluD3* allele *c* genetic background, and the genetic effects of *GluD3* (allele *a* over *c*) can only be detected in the *GluB3* allele *c* genetic background. The results were consistent among the three field trials.

GluD1 and *GluB1* (high molecular weight glutenin subunit) loci have been shown to have highly significant additive genetic effects on dough maximum resistance (Table 3). The effects were positively associated with the Bx17 + By18 allele (relative to Bx7 + By9) and Dx5 + Dy10 (relative to Dx2 + Dy12). In addition, when the analysis was expanded to 900 other molecular

markers, no epistasis was detected between HMW glutenins and other markers for Rmax.

Conclusions that can be made based on these observations are that LMW glutenins are related with dough extensibility, but their genetic effects are conditional upon genetic backgrounds (epistatic interactions). Thus the genetic effects of LMW glutenins may only be detectable in certain genetic backgrounds, causing varying conclusions to be made as to their effects. HMW glutenins are linked to Rmax, with no interactions with any other markers. These results provide further information on the importance of glutenin allelic composition on the quality attributes of wheat lines.

Table 1
Genetic characterisation of *GluB3* on dough extensibility

Trial	<i>d-c</i>		<i>dc-cc</i>		<i>Da-ca</i>	
	a (cm)*	P	a (cm)	P	a (cm)	P
ROS	0.8	7E-6	1.1	3.3E-5	0.45	0.053
ROM	0.7	3.5E-5	1.2	4.1E-6	0.3	0.23
STO	1.3	5.1E-5	1.9	3.3E-6	0.4	0.53

*: additive effects; *dc-cc* means *GluB3d-GluB3c* with *GluD3c* background, *da-ca* means *GluB3d-GluB3c* with *GluD3a* background; *GluB3* alleles: *d* (Cranbrook) and *c* (Halberd), *GluD3* alleles: *a* (Cranbrook) and *c* (Halberd). ROS = Roseworthy, ROM = Roma, STO = Stow.

Table 2
Genetic characterisation of *GluD3* on dough extensibility

Trial	<i>a-c</i>		<i>ac-cc</i>		<i>Ad-cd</i>	
	a (cm)*	P	a (cm)	P	a (cm)	P
ROS	0.3	0.058	0.6	0.011	0	0.65
ROM	0.8	0.0006	1.2	6.5E-8	0.3	0.11
STO	1.6	0.0003	2.2	4.2E-7	0.8	0.09

*: additive effects; *ac-cc* means *GluD3a-GluD3c* with *GluB3c* background, *ad-cd* means *GluD3a-GluD3c* with *GluB3d* background; *GluB3* alleles: *d* (Cranbrook) and *c* (Halberd), *GluD3* alleles: *a* (Cranbrook) and *c* (Halberd). ROS = Roseworthy, ROM = Roma, STO = Stow.

Table 3
Additive effects of *GluB1* and *D1* on Rmax

Locus	Field trial	LOD	%	P	Add
GluB1	ROS	11.01	27	<0.000001	-165.33
	ROM	7.81	20	<0.000001	-56.90
	STO	5.73	15	<0.000001	-113.71
GluD1	ROS	4.23	11	0.00001	107.29
	ROM	10.63	26	<0.000001	65.06
	STO	8.79	22	<0.000001	137.72

% = explained variance, Add = additive effects; ROS = Roseworthy, ROM = Roma, STO = Stow.

Example 2

A comparison between simple interval mapping and composite interval mapping: Software QtlCartograph (Basten et al., 1994; 1997) was used to conduct simple interval mapping and composite interval mapping for the trait dough mixing time. Model VI (Basten et al., 1997) was used to select background markers with a window size of 10 cM. The results (Figures 1 and 2) indicated that simple interval mapping revealed a wide genome region around the *GluB1* locus with a likelihood ratio (LR) value above the significance threshold. It is rather difficult to judge whether there is only one QTL or more than one QTL around the *GluB1* locus. Composite interval mapping narrowed the confidence interval, making it clear that there is only one QTL for mixing time centred on the *GluB1* locus.

Example 3

A coadaptive epistasis for dough mixing time: Dough mixing time measured for the Roma field site material was studied. The results (Figure 2) indicate that two markers on chromosome 5B, PAAC/MCAC234 and PAAC/MCAC1, do not have independent genetic effects on mixing time (LLR = 0.28 and 3.01, respectively, the usual LLR threshold is 6). However, the second marker (PAAC/MCAC1) showed a significant effect (LLR = 9.77) in the genetic background of the Halberd type of the first marker (PAAC/MCAC234). This genetic effect is often neglected without looking at interactions between different loci.

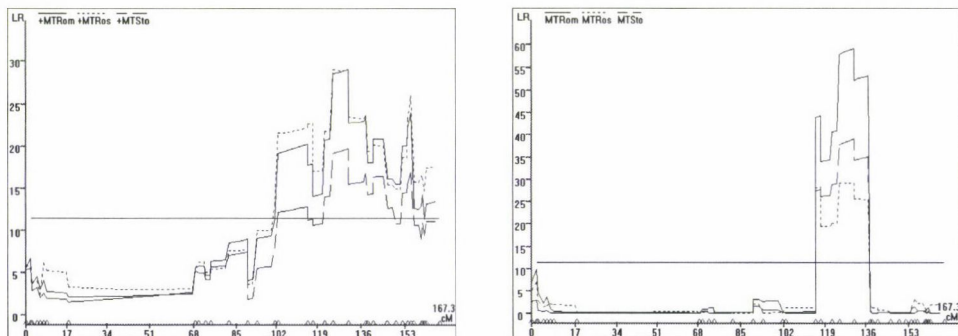


Fig. 1. Simple interval (left) and Composite interval (right) mapping results for dough mixing time on chromosome 1B. X-axis lists LR values, Y-axis represents chromosome 1B starting from short arm (left) to long arm (right). The genetic linkage distance is marked in cM. The *GluB1* locus is located at approximately 128 cM. MTRom = mixing time measured in the Roma field trial, MTRos = mixing time measured in the Roseworthy field trial, MTSto = mixing time measured in the Stow field trial.

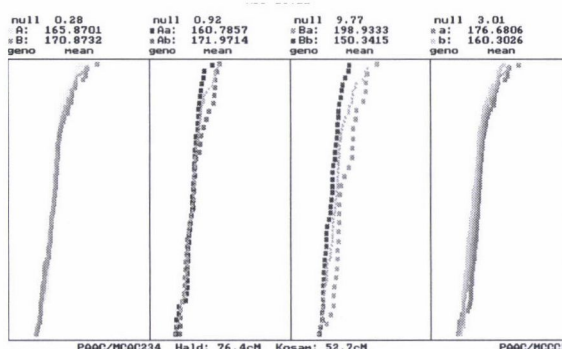


Fig. 2. A coadaptive epistasis for dough mixing time. Epistat display for the trait mixing time in the Roma field trial and the markers PAAC/MCAC234 and PAAC/MCCC1. Cumulative distributions of different genotypic subpopulations are shown in the figure. Panels 1 and 4: the population is subdivided into two genotypes, parent Cranbrook type and Halberd type (A and B for marker PAAC/MCAC234, a and b for marker PAAC/MCCC1). Panels 2 and 3 represent 4 subpopulations according to the individual genotype combination. Above each panel, values of the means for the two genotype sub-populations are shown. The log-likelihood ratio (null LLR, usual positive threshold is 6) is shown for each panel above the means.

Conclusions

Having established the appropriate genetic population, the success of a QTL analysis approach to identify genetic markers linked to important quality traits in wheat relies on 2 factors: our ability to generate a high density molecular map of the population and the extent and accuracy of the quality measurements made for each of the progeny lines for each environment. The former requires the scoring of a large number of polymorphic markers (e.g. 800) for each of the 200 or so progeny lines, or 160,000 marker assays in total. With the current technology used for cereal mapping (e.g. RFLP, SSR or AFLP markers scored by gel electrophoresis) this number of marker assays represents a large investment of time and resources, usually spread over several laboratories over several years. This imposes a significant limitation on the number of different crosses (and thus QTLs) that can be effectively studied. Marker validation in different crosses segregating for the same trait is similarly limited. Millions of single nucleotide polymorphisms (SNPs) have been identified in humans through extensive DNA sequencing projects. These SNPs represent a new class of DNA marker that may be scored in microarray-based formats, which permit tens of thousands of marker assays to be run by a single laboratory per day. In the future, the development of SNP markers and the adoption of the technologies currently under development for the genetic analysis of humans should overcome the current limitations of low throughput and high cost in cereal genetic analysis.

For wheat quality measurements, where a similar investment is required for each population studied, considerable progress has been made in refining and miniaturising many of the testing procedures. However, there is a requirement for the further development of high throughput wheat quality measurement technologies. Unfortunately, it is unlikely that we can rely on advances in other fields to solve the current limitations in the cost and throughput of these tests.

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USE OF PERFECT MARKERS IN WHEAT QUALITY RESEARCH AND BREEDING

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A primary objective of the wheat breeder is to develop populations with optimum combinations of genes controlling key traits. As more is known about the control of specific traits by specific genes, more emphasis can be placed on the use of markers for specific genes, or specific alleles of genes, in wheat breeding. Markers that define specific genes are known as “perfect” markers as they are 100% linked to a gene controlling some significant component of the variability in the trait of interest. Perfect markers are of interest not only because they provide the breeder with a direct means of selecting for a trait, but also because they are directly linked to the mechanism underpinning the trait and are therefore highly valuable from a research perspective. In this paper, we discuss the range of perfect markers available for use in breeding and research in wheat quality. The choice of genes selected, the types of polymorphisms in the genes being targeted, the marker systems used to reveal the polymorphism, and strategies for the implementation of the markers in breeding and research are discussed. In addition, we discuss the future potential for the development of perfect markers given the rapid developments in cereal science and genomics.

Key words: wheat, quality, molecular marker, gene, breeding, PCR

Introduction

Wheat breeders are increasingly using markers as a means of increasing the efficiency of their breeding programmes. Markers provide two general advantages to the breeder. First, they can be used to follow a specific gene in a population, allowing the breeder to make selections for the presence or absence of that gene. The usefulness of a marker is dictated to a large extent by its proximity to the gene underlying the trait under investigation. Secondly, markers can be used to define the background genotype of a population, and this information can be used to make selections and increase overall breeding efficiency. In this case, a suite of polymorphic markers evenly spread over the genome is required. This paper is concerned with the former example, the use of markers to follow specific genes in populations, both for breeding and research purposes.

The development of markers for specific key genes can occur through a number of routes; however, two routes are commonly followed. Firstly, analysis of genetic populations may identify a quantitative trait locus (QTL) that accounts for a high degree of the variation in a particular phenotype, and from the genetic map of the population, a marker tightly linked to that trait (e.g. within 10 cM) identified. In this case, the marker type may be from any one of the technologies used to generate the map, e.g. restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), microsatellite (or simple

sequence repeat, SSR) or single nucleotide polymorphism (SNP). For this approach, a well-developed molecular map of the population is required, representing a substantial research investment. In addition, the polymorphism detected by the linked marker is extremely unlikely to be directly involved in determining the trait and therefore provides no information on the underlying molecular basis of the phenotype. The second route for marker development is to identify the gene or genes underpinning a specific trait on the basis of mechanistic studies, biochemical analysis, candidate gene analysis or through approaches such as map-based cloning. The presence of the gene, or specific alleles of the gene, can then be directly assayed by a number of diagnostic approaches. Such markers are frequently referred to as "perfect" markers as they cannot be separated from the trait of interest through recombination events. Having made this definition of a "perfect" marker, there is one sub-set of markers, frequently encountered in wheat breeding, that are "perfect" markers in that they are 100% linked, but are not located in the target gene. These are markers for alien chromosome segments, such as the barley yellow dwarf virus (BYDV) resistance locus from *Thinopyrum intermedium* (Francki et al., 2001), where recombination between the alien chromatin and the wheat genome is extremely low (Banks et al., 1995) and hence the entire alien segment is effectively transmitted as a non-recombining block. Perfect markers offer substantial advantages over linked markers. Linked markers, such as microsatellites or AFLPs, are only useful if the parents of a new cross are polymorphic for the markers identified in the original population. In addition, implementation of a marker in new germplasm requires verification that the linked marker is in fact linked to the trait of interest. This marker validation is not required for perfect markers, which are applicable to all germplasm sources.

Within the class of perfect markers, where the marker is the casual gene itself, there are two classes of marker. Firstly, those that simply mark for the presence or absence of the gene. This is typical of the situation in disease resistance where the gene being targeted is not present in the acceptor germplasm and the marker is used to drive the introgression of the trait from a donor into the receptor background. Secondly, the perfect marker may be targeted to reveal the presence of a specific allele of a gene in a situation where the target gene is always present, as one allele or another, in both the donor and recipient germplasm. This situation is frequently encountered in breeding for wheat quality where, for example, the high molecular weight glutenin genes are always present in a breeding population but the objective of marker implementation is to select for one allele over another.

The identification of a perfect marker for a specific trait is dependent on the demonstration that a particular gene accounts for a significant amount of the variation in the target trait. While in the past, correlative studies have been used to infer relationships between particular genes and phenotypes, the demonstration of causality is increasingly being done through the analysis of

defined populations, using techniques such as quantitative trait locus (QTL) analysis (Lander and Botstein, 1989). The use of such populations for the analysis of the genetic basis of wheat quality traits has recently been reviewed (Kammholz et al., 2001; Chalmers et al., 2001) and the number of QTLs associated with wheat quality traits is growing (reviewed by Langridge et al., 2001). QTL analysis has confirmed that variation in some important wheat quality traits, for example dough strength and starch swelling power, are associated in a number of populations with loci containing well characterized genes, such as high molecular weight glutenin subunits (HWMGS) and granule bound starch synthase 1 (GBSS1 or *waxy* protein). Recent developments in QTL analysis provide the tools to analyse epistasis (interactions between genes contributing to the observed phenotype) and QTL \times environment interactions (Wang et al., 1999).

In a manner analogous to reverse genetics, the definition of sequence variation in genes, thought to be associated with particular quality traits, such as low molecular weight glutenin subunit (LMWGS) or gliadin genes will allow for the examination of the association of alleles of these genes with specific quality attributes, such as dough extensibility and mixing time.

The presence or absence of particular genes can be assayed at a number of different levels. Genes clearly controlling a particular morphological trait, e.g. grain colour, presence of awns, can be very easily scored across large numbers of individuals in populations. However, the number of genes for which such facile scoring is available is low. Variation in wheat quality features has long been associated with different HMWGS and protein analysis techniques have been used to follow the presence or absence of particular alleles and a very extensive literature exists characterizing the various alleles and methods of scoring by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE, Gupta and MacRitchie, 1991) or by reverse phase HPLC (Burnouf and Bietz, 1984; Marchylo et al., 1989; Sutton, 1991; Margiotta et al., 1993). Similarly, gliadin alleles may be scored by acid-polyacrylamide gel electrophoresis (A-PAGE, Bushuk and Zillman, 1978; Autran et al., 1979; Wrigley et al., 1982; Metakovsky et al., 1984; Metakovsky, 1991). The advantage of protein analysis of glutenin alleles is that a number of genes can be scored in one experiment; the disadvantages are that the method is sensitive to environmental influences, and an assumption is made that proteins of the same electrophoretic mobility are encoded by identical genes. An alternative to direct analysis methods such as electrophoresis is the use of specific antibodies to key proteins. In an elegant example of this method, Gale et al. (2001) developed an antibody specific for a region of the GBSS1 protein encoded by the *WxB1* locus, which discriminates this protein from products of the homoeologous genes on chromosomes 7A and 7D, respectively. This method has the advantage that analysis using ELISA procedures is very efficient and large numbers of samples in a breeding programme can be processed at low cost. The disadvantage of each of these protein analysis methods is that they require seed to be set before the assay can be conducted.

In contrast, DNA-based assays have the advantage that they can be conducted on any tissue, and the results are independent of environmental or management influences on plant growth and development. The explosion of sequence data available for crops such as wheat over the past decade now allows many genes of interest to the breeder to be assayed at the DNA level. A multiplicity of methods for the analysis of polymorphisms in plant DNA have been developed over the years, each with a different acronym, and each with advantages and disadvantages. The major methods that have remained in widespread use and applied to gene mapping and breeding are:

1. RFLP: restriction fragment length polymorphism (Tanksley et al., 1989). This technique was very useful in the early stages of gene mapping in wheat; however, it is slow, requires comparatively large amounts of pure DNA and is not amenable to automation.

2. AFLP; amplified fragment length polymorphism (Vos et al., 1995). A patented method that generates large numbers of anonymous markers efficiently. The major disadvantage of the technique is the low information content of each data point and the difficulty in correlating AFLP markers from one population to another – a very useful technique, however, in the construction of high density maps.

3. Microsatellites; these rely on the presence of repetitive series on di-, tri- or higher order repeat regions in the wheat genome (Tautz and Renz, 1984; Tautz et al., 1986; Weber and May, 1989). The advantage of microsatellites is that the high degree of polymorphism at each site provides an efficient marker system, while there is a high degree of portability of use of microsatellites from one germplasm pool to another. Microsatellites have been extensively used in both mapping and breeding.

4. Perfect markers; PCR-based markers amplifying polymorphic regions from, or immediately adjacent to, target genes. In some instances, such as the introgression of a disease resistance gene from an *Aegilops tauschii* donor, the objective is to score the presence or absence of the gene, and thus the gene or a flanking region can be directly targeted as the hybridisation site of PCR primers. However, in many cases, the information required is the nature of the alleles present in a particular line. A number of strategies have been used in plant breeding to identify useful markers within genes. First, some genes contain microsatellite-like regions – it is estimated that about 2% of wheat expressed sequence tags (ESTs) contain a microsatellite region. These can be targeted as useful sites of allelic variation. Secondly, many genes contain small insertions or deletions that can be targeted. In this respect, the seed storage genes are rich sources of insertion/deletion mutations that can be targeted to generate polymorphic markers. In recent years, large numbers of sequences of members of gene families have been generated and these have proved to be rich sources of polymorphisms, many of which are single nucleotide polymorphisms (SNPs), which can be targeted to generate allele specific (AS-) PCR assays. This

technique utilizes the inefficiency of the extension by Taq polymerase of primers containing a mismatched nucleotide at their 3' end (Ugozzoli and Wallance, 1991; Newton et al., 1989; Drenkard et al., 2000). While the expressed regions of many genes are highly conserved, the introns and flanking regions (promoter regions and 3' untranslated regions) of genes tend to be more polymorphic and therefore rich sources of SNP-based markers.

The past 5 years have seen a dramatic further increase in the number of wheat DNA sequences in the public domain. Cooperative efforts such as the International Triticeae Mapping Initiative (ITMI) and USDA EST programmes have seen the generation of substantial shared resources of expressed sequences in wheat. These sequence collections remain highly useful sources of DNA sequences that can be used to identify perfect markers, particularly allele-specific markers. In addition, concerted attempts to sequence genes at particular loci have been made and these have delivered sets of sequences into public databases. Perfect markers of each of the types listed above can be designed from sequence comparison conducted using these and other resources.

Wheat provides its own particular challenges in the development of perfect markers. In particular, the hexaploid nature of wheat can make the development of allele-specific markers more challenging when there are highly homologous genes at homoeologous loci which might also be amplified. The design of primers which amplify only the target gene from one of the three genomes is frequently possible for highly heterogeneous genes such as the seed storage protein genes (Ahmad, 2000), and is also possible for genes such as the highly conserved genes of the starch biosynthesis pathway (McLauchlan et al., 2001). Many of the genes targeted for the improvement of wheat quality are seed storage protein genes, and in addition to their being present on each of the three genomes, they are present in large, complex gene families. It would be ideal to have the sequence of all of the members of the gene family in order to design highly specific, targeted markers for specific alleles. However, for many gene families (e.g. LMWGS and gliadins) this luxury is currently denied to us and so primers are designed on the basis of the available sequences and tested empirically.

Putative markers for important traits need to be validated across a range of different germplasm sources in order to establish the robust nature of the marker and the validity of relying on the marker in place of a phenotypic test. This could be done through the analysis of the marker in diverse highly defined mapping populations. While this is sometimes possible, it is not always an option. An alternative is to follow the genes in breeding populations to assess the degree of variation that is accounted for by the marked gene. A further alternative is to conduct a retrospective analysis of the impact that a marker might have had in breeding or research populations, if the data are available and can be relied upon.

Once a set of markers have been identified that provide advantages in breeding and genetic research, a final consideration is how to implement the use

of the marker in an efficient and cost-effective manner. Bottlenecks in the implementation of DNA-based markers are (1) having access to a suitable set of validated markers, (2) efficient DNA extraction protocols, (3) multiplexed PCR reactions, (4) efficient, high throughput methods for the analysis of the amplified product. In the remainder of this paper, we present some examples of perfect markers for wheat seed storage protein, starch biosynthesis and grain hardness genes that allow the breeder to control some of the known variability in these important quality-determining components of the grain.

Materials and methods

Wheat DNA extraction

Genomic DNA was extracted from 3–6-day-old hypocotyls of germinating seeds (10 mg) and from 50 mg of flour for the doubled haploid lines using a rapid isolation technique (Stewart and Via, 1993).

PCR analysis

PCR was performed in a reaction volume of 20 µl using 20 ng of wheat genomic DNA, 1 unit HotStar *Taq* DNA polymerase (Qiagen), in 1× PCR buffer (Qiagen, containing 1.5 mM MgCl₂), 200 µM dNTP mix and 10 pmol each of the forward and reverse primers. Amplification conditions for the PCR reaction were 1 cycle of 95°C for 5 mins, followed by 38 cycles of: 95°C for 30 s, 58°C for 30 s and 72°C for 1 min. PCR products were separated in 2% agarose gels and visualised by ethidium bromide staining.

PCR primer design

PCR primers were designed to have annealing temperatures of approximately 58°C using the '4 + 2 rule' (4°C for each G/C, 2°C for each A/T nucleotide). For gene-specific PC primers, Blast N alignments (NCBI) were used to identify unique regions. For gene and allele-specific PCR primer design, polymorphic nucleotides were placed at the 3' end of one or both primers to maximize the selectivity of the PCR assays.

PCR Primers (5' to 3')

HMWGS Dx5 allele-specific (Ma et al., 2002)

forward: CGTCCCTATAAAAGCCTAGTT

reverse: AGTATGAAACCTGCTGCGGAC

product size: 478 bp

GBSS Wx-B1 homoeoallele-specific PCR (from the sequence of Murai et al., 1999)

Forward: CCCC GAAGCAACAAAGCCG

Reverse: GACCGTTGGCCTGCAGAC

Product size: 295 bp

Puroindoline 'a' gene-specific PCR (from the sequence of Gautier et al., 1994)

Forward: ATTCATCTCCACCTGCACC

Reverse: ACACGCAGTGGTATGTGAC

Product size: 533 bp

Puroindoline 'b' D1b allele-specific PCR (from the sequence of Giroux and Morris, 1997)

Forward: CCCACAAAATGGTGGAAGA

Reverse: GAACACAGATCAATATACAAGG

Product size: 356 bp

Results

Dough properties

The high molecular weight glutenin subunits (HMWGS) encoded by the Glu-1D locus have been demonstrated to have a major impact on the strength of doughs prepared from wheat flour. Two genes are encoded at this locus, an “x” and a “y” type subunit. Higher dough strength has been associated with the combination of alleles 5x and 10y. In Figure 1, a perfect marker for allele 5x is shown, which can be used in a breeding programme as a dominant marker for increased dough strength.

Starch properties

The granule bound starch synthase genes on chromosomes 7D and 4A encode starch synthases present only in the starch granule of wheat. These genes have been demonstrated to be absolutely required for amylose synthesis through the creation of “waxy” wheats containing no amylose (Nakamura et al., 1995; Zhao and Sharp, 1998). However, single and double nulls of GBSS have interesting technological properties and there is strong interest in breeding programmes for the selection of null or normal GBSS alleles at each of the three homoeologous loci (Zhao et al., 1998). In Figure 2, an example of a perfect marker for the GBSS gene encoded on chromosome 4A is shown, allowing the rapid screening of breeding material for homozygous null lines.

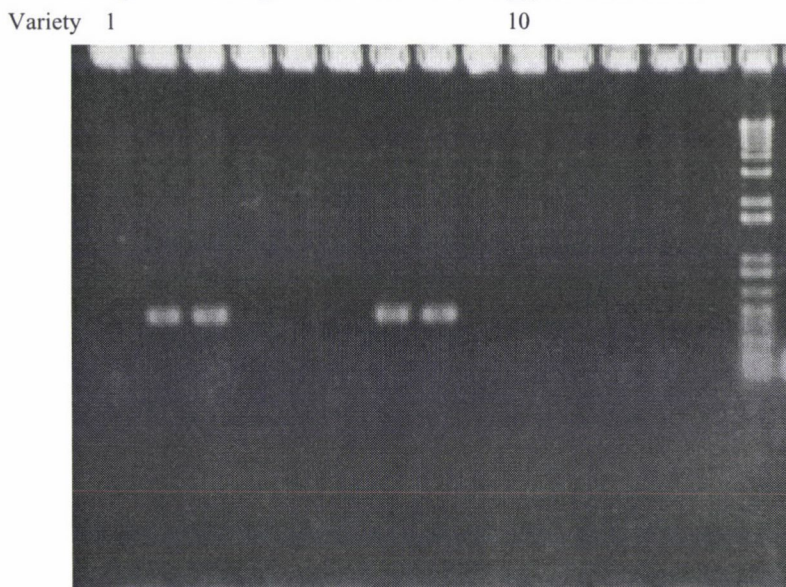


Fig. 1. Allele-specific PCR for HMWGS Dx5. Fourteen commercial cultivars containing either the Dx5 + Dy10 (Glu-D1'd') allelic pair or Dx2 + Dy12 (GluD1'a') allelic HMWGS pair were analysed. Only cultivars containing the Dx5 gene produced the 478 bp PCR product. Products were electrophoresed in 2% agarose and stained with ethidium bromide. The 1 Kb DNA ladder (BRL) was used as marker.

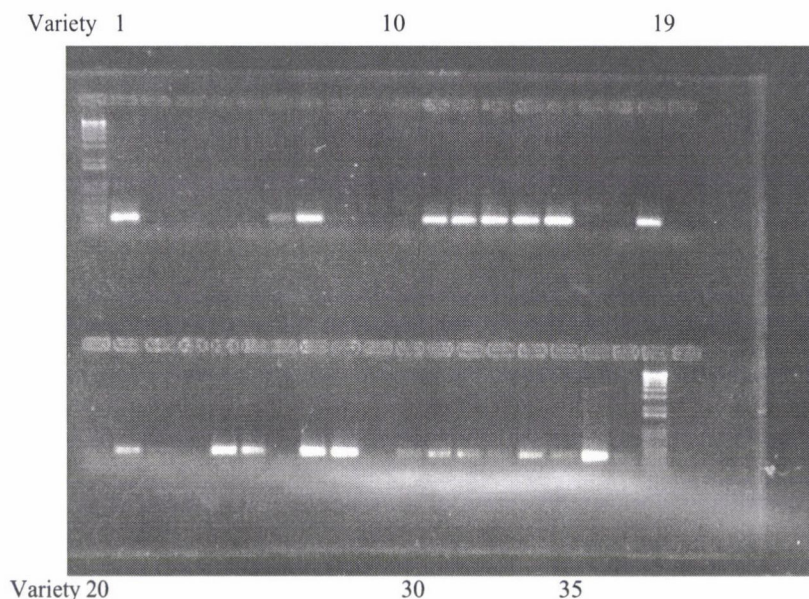


Fig. 2. Detection of the presence of the Wx-B1 allele of the GBSS1 gene in commercial wheat cultivars using PCR primers specific for this homoeoallele. PCR products were electrophoresed in 2% agarose and stained with ethidium bromide. The 1 Kb DNA ladder (BRL) was used as marker. Positive lanes represent varieties that are classified as 'Wx-B1a' (gene present) whereas negative lanes represent varieties that are classified as 'Wx-B1b' (null for Wx-B1, also known as 'GBSS 4A null').

Grain hardness

The puroindoline 'a' and puroindoline 'b' genes have been associated with the control of grain hardness in wheat. While the proof that they directly cause differences in grain hardness is inconclusive, they are certainly located at the grain hardness locus and are very tightly linked to the phenotype. In Figures 3 and 4, examples of dominant markers for puroindoline 'a' and puroindoline 'b', respectively, are shown. The presence of a product from the puroindoline 'a' PCR test does not discriminate between hard and soft lines. However, in our experience lines that are negative for the marker are always hard. In contrast, a positive result for the puroindoline 'b' marker provides strong evidence that a line is hard and negative lanes contain other alleles of the gene and may be classified as hard or soft. The use of the two markers in combination in germplasm segregating for the target alleles will allow the rapid identification of lines with the target hardness.

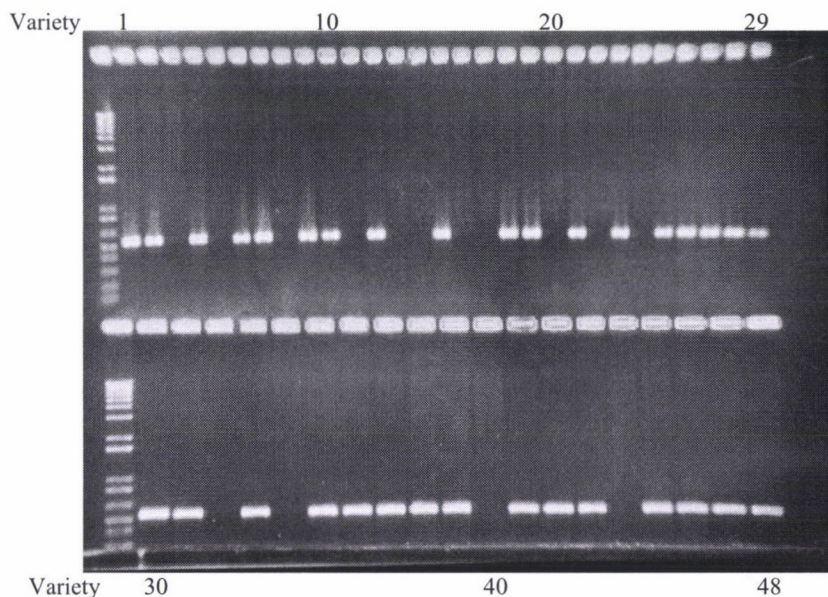


Fig. 3. Detection of the presence of the puroindoline 'a' gene in commercial wheat cultivars using PCR primers specific for this gene. PCR products were electrophoresed in 2% agarose and stained with ethidium bromide. The 1 Kb DNA ladder (BRL) was used as marker. Negative lanes represent hard-textured varieties that are null for this gene. Positive lanes contain the gene and may be classified as hard or soft.

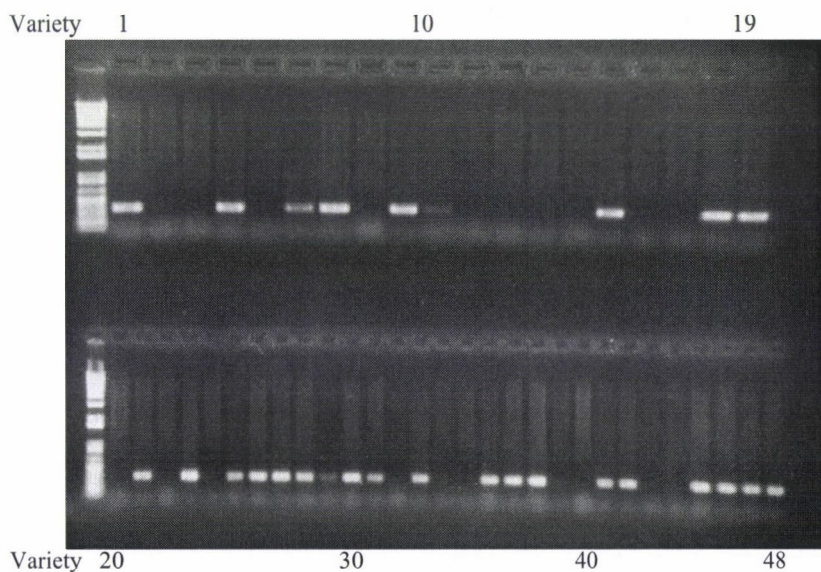


Fig. 4. Detection of the presence of the puroindoline 'b' D1 b allele in commercial wheat cultivars using PCR primers specific for this allelic variant of the gene. PCR products were electrophoresed in 2% agarose and stained with ethidium bromide. The 1 Kb DNA ladder (BRL) was used as marker. Positive lanes represent hard-textured varieties that contain the pin b D1 b allele of this gene. Negative lanes contain other alleles of the gene and may be classified as hard or soft.

Discussion

The generation of large amounts of gene sequence data for wheat provides the promise of greatly expanding our capacity to develop perfect markers that allow genes, and alleles of genes, to be followed in breeding or research populations. This will be important to the efficiency of those programmes but also holds out the promise of allowing us to gain much greater insights into the roles of those genes, and their interaction with other genes in the genetic background. In addition to these benefits, molecular markers will be important in two areas of the seed industry; firstly, in assisting in the ownership and registration of varieties of wheat, and secondly, in quality assurance where producers and customers may want to confirm the identity of genetic material. This will become important as specialized proprietary lines of wheat are developed for specific end-use applications.

The future use of perfect markers in wheat will depend on a range of factors, including the following:

1. A number of key quality, agronomic and disease resistance genes have yet to be defined, and therefore perfect markers for these genes cannot be developed. The continuing pace of research aimed at identifying these key genes means that within a further few years, many more important genes are likely to be identified and characterized.

2. Sequencing efforts and databases continue to grow, providing rich new sources of data from which markers for key genes and their alleles can be designed. For example, the rice genome has now been sequenced in its entirety.

3. Improved implementation platforms will be developed that allow the breeder and researcher to make use of the markers that are developed. An important likely development is that of highly parallel systems for marker scoring, potentially based on SNP technologies developed for mammalian research and human diagnostics.

The development of perfect markers in wheat is a very exciting development in both breeding and research, allowing the efficient genotyping of key traits in key populations. This will undoubtedly lead to new knowledge of the roles of these and other genes, and provide a platform for the development of wheats with improved agronomic and quality characteristics.

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MARKER-ASSISTED BREEDING FOR THE IMPROVEMENT OF DISEASE RESISTANCE IN CEREAL CROPS

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Recent reviews on molecular markers developed for wheat genes have been published by Langridge and Chalmers (1998) and Gupta et al. (1999), while updated lists are maintained in the catalogue of gene symbols for wheat (<http://wheat.pw.usda.gov/ggpages/pubs.shtml>).

Key words: morphological marker, isozymes, RFLP, PCR, DNA arrays, cereals, disease resistance

Molecular markers for disease resistance genes in cereals

Morphological markers

The association of certain morphological characters with other traits was recognized a long time ago (e.g. Sax, 1923). In wheat resistance breeding, for instance, the association between leaf tip necrosis and the *Lr34* gene conferring adult plant leaf rust resistance has found some application, for example in the CIMMYT breeding programme (Singh, 1992).

Biochemical markers (isozymes)

Resistance against eyespot (*Pseudocercospora herpotrichoides*) in wheat cv. VPM1 and its derivative varieties Roazon and Rendezvous is conferred by a single dominant gene (*Pch1*) derived from *Aegilops ventricosa*. The gene is located on chromosome 7D and closely linked to a marker gene for endopeptidase (*Ep-D1b*) which has been used for routine resistance screening in several breeding programmes (Worland et al., 1988; Vahl and Muller, 1991).

DNA hybridisation markers (RFLP)

The first framework molecular maps in cereals were based mainly on restriction fragment length polymorphism (RFLP) markers and a range of genes including resistance genes could be mapped. One well-known example is the mapping of the *Mla* gene conferring powdery mildew resistance in barley (Jahoor et al., 1993), where with certain RFLP probes several alleles of the *Mla* locus could be discriminated. A range of other resistance genes were mapped with RFLPs, for example resistance to powdery mildew in wheat (Hartl et al., 1993; 1995) and many other resistance genes (see Langridge and Chalmers, 1998 for further references).

Despite the fact that the first useful molecular maps in cereals were based on RFLPs, their application in breeding has remained limited. The main reason appears to be the technical complexity and high cost of the RFLP method. It requires large amounts of high quality DNA and involves either radioactivity or complex biochemistry for detection.

Polymerase chain reaction (PCR)

The availability of PCR-based markers has strongly enhanced the applicability of marker-assisted selection (MAS). For the rapid development of high resolution molecular maps at reasonable cost the amplified fragment length polymorphism (AFLP) technique has great potential (Vos et al., 1995). AFLP does not require prior knowledge of the genome one is working with. The problem with AFLPs is that they are not really user-friendly markers to apply, as they are technically fairly complex to perform and show a multiplex fragment pattern and are therefore difficult to analyse. Random amplified polymorphic DNA (RAPD) markers are technically easy to perform but suffer from limited reproducibility.

Specific PCR assays offer the most promising potential in MAS. Specific PCR assays may be derived by conversion of other markers (RAPD, AFLP, RFLP). This usually involves cloning and sequencing of e.g. RAPD or AFLP bands. By designing appropriate primer pairs STS (sequence tagged site) or SCAR (sequence characterised amplified regions) assays can be developed. PCR fragments of similar size but with internal sequence differences may be cleaved with a restriction enzyme and thus polymorphism can be visualized (CAPS marker: cleaved amplified polymorphic sequence). More sophisticated assays to detect single nucleotide polymorphisms (SNP) have become available in recent years and will be available for high throughput analysis.

The development of a large number of SSR (microsatellite) markers (Bryan et al., 1997; Roder et al., 1998; Song et al., 2001; Eujayl et al., 2002) has opened the way to using these in breeding applications without the need of marker conversion, provided they are tightly linked to the genes of interest. SSRs are technically easy to perform and are amenable to automatization and high throughput applications. Examples of resistance genes in wheat and barley mapped with PCR assays are presented in Table 1.

For almost all resistances mapped so far, markers are more or less tightly linked to the genes of interest, but do not detect the genes themselves. With the novel methods of genomics the actual resistance genes may become accessible in the near future, thus allowing the development of diagnostic assays.

DNA arrays

The latest advent in marker technology is represented by DNA arrays, which would allow the simultaneous detection of thousands of loci in a plant in a parallel assay. Whether or not such arrays will find application in plant breeding programmes remains to be seen.

Table 1
Examples of PCR assays for cereal resistance genes (non-exhaustive list)

Crop	Disease	Resistance gene	Reference
Barley	BYMV	<i>rym4</i>	Turesson et al., 1998; Ordon et al., 1999
Barley	BYMV	<i>rym4, rym5</i>	Graner et al., 1999
Barley	BYMV	<i>rym9</i>	Ordon et al., 1999; Werner et al., 2000
Barley	stem rust	<i>Rpg1</i>	Horvath et al., 1995; Penner et al., 1995
Barley	leaf rust	<i>Rph16</i>	Ivancic et al., 1998
Barley	leaf rust	<i>Rph9, Rph12</i>	Borovkova et al., 1998
Wheat	WSMY	<i>Wsm1</i>	Talbert et al., 1996
Wheat	BYDV	<i>Th. intermedium</i>	Ayala et al., 2001
Wheat	loose smut	<i>T10</i>	Procunier et al., 1997
Wheat	common bunt	<i>Bt-10</i>	Laroche et al., 2000
Wheat	leaf rust	<i>Lr1</i>	Feuillet et al., 1995
Wheat	leaf rust	<i>Lr9</i>	Schachermayr et al., 1994
Wheat	leaf rust	<i>Lr24</i>	Schachermayr et al., 1995
Wheat	leaf rust	<i>Lr28</i>	Naik et al., 1998
Wheat	leaf rust	<i>Lr35</i>	Seyfarth et al., 1999
Wheat	rusts	<i>Lr37, Yr17, Sr38</i>	Robert et al., 1999
Wheat	leaf rust	<i>Lr39</i>	Raupp et al., 2001
Wheat	leaf rust	<i>Lr47</i>	Helguera et al., 2000
Wheat	powdery mildew	<i>Pm1c</i>	Hartl et al., 1999
Wheat	powdery mildew	<i>Pm13</i>	Cenci et al., 1999
Wheat	powdery mildew	<i>Pm21</i>	Liu et al., 1999
Wheat	powdery mildew	<i>Pm24</i>	Huang et al., 2000
Wheat	powdery mildew	<i>Pm27</i>	Javre et al., 2000
Wheat	powdery mildew	<i>Pm29</i>	Hartl, pers. comm.

Two examples of marker-assisted selection in cereal resistance breeding

Example 1: Breeding for resistance to the barley yellow mosaic virus (BYMV) complex

The BYMV complex is a major problem in winter barley in many parts of Europe. The disease was first reported in Europe in 1987/88, but was discovered in Japan over 50 years ago. In Europe the complex consists of at least 3 strains: BaMMV, BaYMV, BaYMV-2, which belong to the group of potyviruses and are transmitted by the soil fungus *Polymyxa graminis* (Turesson et al., 1998; Ordon et al., 1999)

The BYMV virus complex has caused serious damage in central and western European winter barley crops. In Austria the virus has not been confirmed up to now. However, Austrian winter barley breeding has a strong interest to incorporate BYMV resistance into the new breeding lines. Phenotypic selection for resistance cannot be performed in Austria, so the lines have to be tested in special disease nurseries in France or Germany, which causes some logistic problems. Secondly, phenotypic evaluation for BYDV resistance by sowing in virus-infested fields may also be biased by environmental conditions.

In winter barley breeding the application of DNA markers in BYMV resistance selection is therefore meaningful. A very promising PCR marker is linked to the *ym4/ym5* resistance locus on chromosome 3H of barley. The resistance gene *rym4* was derived from the Dalmatian land race Ragusa and the resistance gene *rym5* from the Japanese line Mokusekko3 (Graner et al., 1999). The SSR marker Bmac29 is closely linked (about 1 cM) to these resistance genes. Lines with the *rym4* allele produced a 158 bp fragment, lines with the *rym5* allele a 160 bp fragment and susceptible lines a 176 bp fragment (Graner et al., 1999). During the application of this marker in Austria it was discovered that several susceptible lines from the Austrian gene pool also showed the 158 bp fragment expected for *rym4* lines. It was discovered that unfortunately *rym4* lines were used in the crossing programme many years ago and a recombination event between the Bmac29 SSR marker and the resistance gene must have occurred. Therefore, selection for *rym4* resistance is not easily possible with the Bmac29 marker in this breeding programme. However, the coupling between the 158 bp Bmac29 allele and *rym5* was still valid. We are therefore now concentrating on the introgression of the *rym5* allele into adapted winter barley material by a combination of doubled haploid breeding and marker-assisted selection. Possibly the main disadvantage of the Bmac29 marker is that it is not freely available.

Example 2: Breeding for Fusarium head blight (FHB) resistance in wheat

FHB, caused by pathogenic fungi of the group *Fusarium*, is a disease which occurs only sporadically in Austria, but may have devastating consequences, as can be seen from the 1993 epidemic in the US and Canada (McMullen et al., 1997).

The Chinese wheat cultivar Sumai3 and its derivatives have proved to possess a high level of stable FHB resistance. The line unfortunately has many agronomic shortcomings and breeders are very reluctant to use it in their routine breeding programmes. Therefore some pre-breeding is necessary to incorporate FHB resistance into more adapted winter wheat material. By conventional crossing and phenotypic selection such a programme may be very slow in progress. Despite the fact that resistance to FHB in Sumai3 was considered to be of quantitative nature, a few quantitative trait loci (QTL) with fairly large effects were detected (Anderson et al., 2001; Kolb et al., 2001). Our own research led to the identification of two major QTLs, located on chromosomes 3B and 5A, respectively (Burstmayr et al., 2002). Fortunately in both QTL regions several SSR markers could be identified. We are now using these markers in a marker-assisted backcross procedure to verify the effect of the QTL in different winter wheat backgrounds and simultaneously develop agronomically adapted breeding lines with acceptable levels of FHB resistance.

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COMPARATIVE MAPPING AND QTL ANALYSIS OF EARLY SPRING ADAPTATION TRAITS IN BARLEY (*HORDEUM VULGARE* L.)

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The possibilities latent in molecular marker-based QTL analyses are presented through the example of studying winter survival and heading date in barley (*Hordeum vulgare* L.). The whole range of QTL experiments consists of several important steps, through which answers are found to the following questions: (1) How many QTLs are involved and where do they map, (2) How does the environment influence the effect of a QTL region (environment \times QTL interactions), (3) When and where are the genes determining the given trait expressed (QTL dynamics), (4) What interactions occur between these QTLs and pathways leading to specific phenotypes, and (5) How consistent is the effect of a QTL region in different genetic backgrounds and in a wider range of germplasms (comparative mapping and association studies)? This knowledge then makes it possible to continue these experiments in the direction of marker-assisted selection and/or gene isolation through marker saturation of the relevant chromosomal regions and map-based cloning. The latter can give an insight into the exact mechanism through which the gene determines the phenotype.

Key words: QTL analysis, comparative mapping, barley, heading date

Introduction

Most of the agronomic traits of crop plants are inherited quantitatively. Their common characters lie in their polygenic control and in the significant effect of the environment, which together determine the phenotype through their interactions. Thus, studying the genetic mechanisms of such traits represented an almost impossible task before the application of molecular markers began. Several biometric methods existed to predict the number of loci involved in the genetic control, but they were based on simplifying assumptions and in addition they could not give answers as to the chromosomal locations of these loci (Lande, 1981). Gene identification was mostly narrowed to qualitative traits with very few exceptions (Takahashi and Yasuda, 1970). From the practical aspect, to make the selection advantage based on phenotypic characterisation more certain, plant breeders are often forced to use replicated trials not only under different environments but also over several years.

For the above reasons, the application of molecular markers and marker-based maps holds great promise both in genetic studies on quantitative traits (Marquez-Cedillo et al., 2001) and in selection for and pyramiding of favourable alleles (Oziel et al., 1996). In addition, markers may represent a starting point in genemap-based gene cloning (Wing et al., 1994).

An example of the QTL study approach is given in this article based on results acquired in the field of winter survival and heading date in barley.

Winter survival and heading date in cereals is the final result of a number of interacting characters that include vernalization response, photoperiod sensitivity, and earliness *per se*. Each of these components is under quantitative control, thus hindering genetic studies. Molecular markers represent good tools for dissecting these complex mechanisms. The chromosomal location and QTL dynamics of major loci contributing to heading date were determined in a spring \times winter barley mapping population. For the better understanding of the genetic control of winter survival and heading date, these characters were also studied in a winter barley genetic background, applying comparative mapping. In addition, a wider range of barley germ plasm of different geographic origin was included in association studies to predict their effect on phenotype and the frequencies with which the specific allelic versions occur in barley accessions.

When a QTL study is carried out, it is important to answer the questions listed below.

How many QTLs are involved and where do they map in the genome?

Classical genetic studies revealed that at least three loci are responsible for growth habit in barley (Takahashi and Yasuda, 1970). They are the *sh*, *Sh2* and *sh3* loci on chromosomes 4, 7 and 5, respectively. Allelic series at each locus are probably present for *Sh2* but also for *sh* (Laurie et al., 1995). In addition, several "earliness *per se*" loci were identified (Gallagher et al., 1991). They are thought to influence flowering time, when plants are fully vernalized and they are independent of the daylength regime. Low temperature tolerance was hypothesized to be a complex, polygenic trait.

Molecular marker-based studies made it possible to identify the chromosomal location of several of the above-mentioned loci, and to map new loci for photoperiod sensitivity and low temperature tolerance (Hayes et al., 1993; Laurie et al., 1994; 1995). The extensive mapping and QTL studies carried out in *Dicktoo* \times *Morex*, a winter \times spring barley population, made it possible to locate major QTLs determining winterhardiness-related traits (Pan et al., 1994). *Dicktoo* is frost tolerant, it has no vernalization response and it is photoperiod sensitive. *Morex* has no low temperature tolerance, no vernalization response and is photoperiod insensitive. In their mapping population more than one QTL region were identified for each of the traits studied, with the exception of winter survival. Five QTLs were determinant of heading date under field conditions, on chromosomes 7H(1), 2H, 3H, two distinct regions of 1H(5) and 5H(7), explaining 66% of the variance altogether, the effect of individual loci being between 11–20%. On these same chromosome regions were located QTLs for heading date under different daylength regimes in a controlled environment, their significance depending on the photoperiod. Under long daylength regimes, chromosomes 2H and 5H(7) were the significant determinants of heading date,

irrespective of the vernalization treatment. Under short daylength, chromosomes 7H(1), 3H and two regions of 1H(5) had a significant effect in the vernalized treatment ($R^2=0.59$), while chromosome 5H(7) was also involved in the unvernallized treatment ($R^2=0.64$). The only significant locus for winter survival was located on chromosome 5H(7), explaining 31% to 79% of the phenotypic variance alone under different environments. This QTL was located in the same chromosome region where the heading date QTL was identified.

These results underline the quantitative nature of the component traits of winter hardiness and early adaptation. After identifying the number and location of QTLs the next important question is how this information can be used. Some of the QTLs have little significance for application in genetic and/or practical research. To distinguish them it is also important to evaluate the stability of the QTLs over different environments and different genetic backgrounds.

How does the environment influence the effect of a QTL region?

QTL studies have revealed that the effect of the environment on QTL regions may also be significant (Hayes et al., 1993). There can basically be three different types of interactions: (1) the significant effect of a QTL is only apparent in one or few of the environments, (2) the QTL is significant in most or all of the environments with a change only in its magnitude, and (3) the QTL is significant but there is a shift in the favourable allele phase among the environments. Knowledge of the type of interaction is important from both theoretical and practical aspects. A change in the favourable allele phase represents a major drawback in utilising the QTL information, while in the case of changes in magnitude the only necessary decision to make is, whether the QTL has a sufficient effect on the phenotype to warrant selection.

QTL \times E due to changes in the magnitude of the QTL effect characterized most of the interactions in most of the experiments (Thomas et al., 1995; Marquez-Cedillo et al., 2001).

In the present case the environment effect was examined by comparing the heading date in the greenhouse and in growth chambers. Our major aim was to dissect the components of heading date and to study the mechanisms of heading date QTLs identified in the *Dicktoo* \times *Morex* mapping population. For this purpose a subpopulation of 19 DH lines was selected from the full population of 100 lines. A detailed photoperiod experiment consisting of seven photoperiod regimes (24, 18, 16, 14, 12, 10 and 8 hours) was carried out on this subpopulation in unvernallised and vernalised treatments. In the case of QTL analyses the appropriate population size is a crucial point. Generally, at least 100 lines are considered to be the minimum number for establishing the QTL effects correctly. Because of the small number of lines the first important task was to compare the QTL results of the full population and the subpopulation, and only when the association was proved could the analysis be continued. Good correlations were found between the results, especially under long daylength (Pan et al., 1994; Karsai et al., 1997a), while under short daylength the

correspondence varied with the chromosomal regions (Table 1). In the case of chromosomes 3H and 1H(5) not only the marker intervals were the same, but also the ratio of the phenotypic variance explained by the QTL, though their LOD scores in the subpopulation did not always reach the significance level usually applied in QTL studies. There was a marker interval shift on chromosome 7H(1), while a new QTL locus with a minor effect appeared on chromosome 4H in the subpopulation.

There are several theories to explain the QTL \times E interactions. According to Paterson et al. (1991) a good association can be established between the general and specific combining abilities predicted from diallel analyses and the QTL \times E interactions. QTLs significant over a wide range of environments are primary determinants of the given traits, such as general combining ability. The QTLs unique to one or a few environments result from the specific conditions existing in the given environment and as such they are parallel to specific combining ability. These single QTLs may be transient regions, detectable only under specific conditions of plant growth or environmental factors, which cannot normally be measured. It is also probable that these single QTLs are not real determinants of the given trait. In the case of heading date, for example, an abiotic or biotic stress apparent in one environment may cause a significant change in heading. If the lines differ in their resistance, this may result in coincident heading date QTLs at the loci responsible for the resistance.

When and where are the genes determining the given trait expressed?

It is a great challenge to explain the mechanisms of the QTL determining the given trait, which serves as the basis for both theoretical and practical applications of the QTL information. To do so there are several possibilities: (1) examining the probability of a coincidence between a QTL and a gene of known chromosomal location (Bezant et al., 1996), (2) studying the QTL dynamics during changes in environmental factors (Karsai et al., 1997a), and (3) separating the trait into its components and carrying out a parallel QTL study involving the trait and its components (Karsai et al., 1997a).

Table 1

Heading date QTL of vernalized plants under short (8 h) photoperiod regime in the full Dicktoo \times Morex population and in a subpopulation of 19 lines studied in a detailed photoperiod experiment in two different environments

Marker intervals	Chromosome location	R ² (%) of full population (Corvallis)	R ² (%) of selected lines (Martonvásár)
Crh1-ABC461	7H(1)	16.0	—
Brz-ABC158	7H(1)	—	17.7
ABG4-ABC174	3H	13.0	19.0
HorD-saflp164	1H(5)	40.0	40.9
IPgd-BCD265c	1H(5)	19.0	23.3
Dhn6-BCD165a	4H	—	15.0

The detailed photoperiod regime experiment carried out in the *Dicktoo* \times *Morex* population made it possible to examine the effect of vernalization response and to evaluate the photoperiod sensitivity of the lines (Karsai et al., 1997a). The heading date QTL on chromosome 5H(7) was found to be relatively independent of photoperiod, while more pronounced in unvernallized treatments. This chromosome region was a more significant determinant of the first node appearance – the indirect indicator of the turning point from vegetative to generative growth – than of heading date, the winter barley allele resulting in delayed development in both developmental phases. Based on these results and those of classical genetics, it became evident that *Sh2*, one of the growth habit loci, contributed to the heading date.

By carrying out a detailed experiment it became possible to calculate the photoperiod sensitivity of each line based on the method of Roberts et al. (1988). The QTL analysis of this value revealed that only one chromosome region played a significant role in determining photoperiod sensitivity, explaining 80% of the phenotypic variance. This region was on chromosome 2H at the same interval where the major heading date QTL was identified. Their LOD value curves also showed a strong similarity, establishing the fact that a locus for photoperiod sensitivity (*Ppd-H1*) was responsible for the heading date QTL in this chromosome region (Karsai et al., 1997a). Laurie et al. (1994) reported similar results. The effect of the *Ppd-H1* locus was strongly dependent on photoperiod; it became a significant determinant of heading date when the daylength was 14 hours or longer. Under these circumstances the developmental rate of lines carrying the sensitivity allele (derived from the winter barley parent) was quickened, resulting in earlier flowering.

The dynamics of heading date QTLs apparent in the *Dicktoo* \times *Morex* population under short daylength was also evaluated (Karsai et al., 2000). In three of the four chromosome regions the winter barley allele significantly delayed plant development, the only exception being the chromosome 4H locus, where the spring barley allele resulted in later development (Table 2). There was a marked difference in the dynamics of these QTL regions in the different vernalization and photoperiod treatments. QTLs determining plant development on chromosome 3H were only apparent in the vernalized treatment. In the case of first node appearance this region became significant in the 12-hour photoperiod regime and reached its maximum effect in the 10- and 8-hour photoperiod regimes, explaining 21.9, 35.2 and 29.9% of phenotypic variation, respectively. The same region determined heading date only in the 10- and 8-hour photoperiod regimes, with r^2 values of 24.9 and 19.2%, respectively. The effect of chromosome 4H was also more pronounced for first node appearance than for flowering. At this developmental phase it became significant in the 12-h photoperiod ($r^2=0.23$), and it determined first node appearance independently of vernalization under daylength shorter than 12 h. Of the two regions on chromosome 1H(5) the one close to *HorD* had the strongest effect on heading date among all the QTL regions. Its effect increased with shortening daylength and was more pronounced in unvernallized treatments. The other QTL, close to *BCD265c*, was significant only in vernalized treatments and when the daylength

was shorter than 10 hours. Comparing these results with those of classical genetic studies and other marker-based research it is probable that we identified the effect of the earliness *per se* locus *ea_{sp}* on chromosome 3H (Gallagher et al., 1991). On chromosome 1H(5) the region of *HorD* may correspond to the effect of *Ppd-H2* (Laurie et al., 1995), while the QTL at BCD265c may be the effect of the *sh3* vernalization response locus (Takahashi and Yasuda, 1970).

What interactions exist between these QTLs and the pathways leading to specific phenotypes?

The physical mapping of the chromosomes revealed that molecular marker-based linkage maps contain strong distortions. The lack of recombination in the centromeric region has been long recognized. When studying deletion lines it also became evident that on each chromosome there are regions known as "hot spots" having much higher recombination frequencies than the average (Serizawa et al., 2001). Thus, long regions of linkage maps may represent in reality a very short part on the physical map. It is a frequent finding in QTL studies that the QTLs of several different traits are located in the same chromosome interval (Hayes et al., 1993; Marquez-Cedillo et al., 2001). Due to the distortion in the linkage maps and the distance among markers it is difficult in some cases to decide whether these overlapping QTLs are the results of tight linkage among different genes or whether they originate from the pleiotropic effects of one gene. A decision will need background knowledge and specific plant materials such as near-isogenic lines. A good example of this approach is represented by the work of Galiba et al. (1995). In wheat the loci of vernalization response (*Vrn1*) and frost tolerance (*Fr1*) were located to the same chromosome region on 5A (Sutka and Snape, 1989), as in barley. Special recombinant lines for the substituted 5A chromosome were studied to prove that this result was due to tight linkage between two different loci.

Table 2

Weight of QTL peaks on first node appearance and heading date in the subpopulation of Dicktoo × Morex under shortening daylength regimes based on linear regression of single markers

Marker	Chromosome	12 hours		10 hours		8 hours	
		uv	vern	uv	vern	uv	vern
First node appearance							
ABC174	3H		4.6D [†]		8.8D		8.2D
Dhn6	4H	18.4M		22.2M	8.0M	24.2M	8.9M
Saflp164	1H(5)		5.4D		8.3D	21.1D	8.4D
BCD265c	1H(5)				9.9D		12.1D
Heading date							
ABC174	3H				13.9D		40.2D
Dhn6	4H				14.8M		35.5M
Saflp164	1H(5)			41.1D	12.9D	60.7D	49.6D
BCD265c	1H(5)				15.7D		40.9D

[†] difference between the parental alleles in reaching the given developmental phase (in days); the letter suffix indicates the allele giving the larger value; D = Dicktoo, M = Morex; uv = unvernallized; vern = vernalized

From breeding aspects it is important to know whether pleiotropy or tight linkage is responsible for the coincident QTL peaks, especially in cases where the favourable alleles of the linked traits originate from different parents. In the case of tight linkage large population size and more detailed phenotypic characterization are needed to identify the very few correlation-breaking individuals.

Several QTL studies have dealt with the pleiotropic effect of a given QTL region (Bezant et al., 1996). Karsai et al. (1997a; 1999) found that both the *Ppd-H1* and *Sh2* loci significantly influenced several other agronomic traits through pleiotropic interactions, including plant height, number of tillers and certain yield components. In the case of *Ppd-H1* on chromosome 2H, QTLs for the node number and grain number of the main tiller, and for thousand-kernel weight were also located in the same region. In photoperiod-sensitive lines long photoperiod regimes result in a quickening of the developmental rate and in the earlier formation of the thermal spikelet. For this reason, they form fewer nodes in the main stem and a smaller number of spikelets with fewer grains in the ear. On the other hand, their larger thousand kernel weights compensate for the lower grain number and there is thus no plant yield QTL connected with the *Ppd-H1* locus.

Loci from independent linkage groups may also modify each other's effect significantly. The easiest way of studying this kind of epistatic interaction between two QTL regions is to compare the phenotypic averages of the four possible marker groups. When the average values of the heterozygotic groups, combining QTL alleles from both parents, differ significantly from the homozygotic groups, containing QTL alleles from one parent only, this indicates an epistatic interaction between the two QTL loci. In this way it was proved that an epistatic interaction exists between the *Ppd-H1* locus on chromosome 2H and the *Sh2* locus on chromosome 5H(7), giving an explanation for the transgressive forms (Pan et al., 1994). Epistatic interaction also served as an explanation for the difference found between the heading date QTL of the full *Dicktoo* × *Morex* population and for the subpopulation of 19 lines (Table 1). The QTL region on chromosome 4H was not a significant determinant of heading date under short daylength in the full population. When the possibility of epistasis between this chromosome region and those of *Ppd-H1* and *Sh2* was examined, *Sh2* was found to have a significant epistatic effect on the 4H locus. When the full population was separated into two groups based on the allele composition at *Sh2* (chromosome 5H(7)), the effect of chromosome 4H on heading date became significant, explaining 17.1% of the phenotypic variance in the case of lines carrying the *Dicktoo* allele at the *Sh2* locus. The average heading date values for the DD, DM, MD and MM groups, based on *Sh2* and chromosome 4H, were 107.7, 115.3, 109.8 and 108.9 days, respectively.

How consistent is the effect of a QTL region in different genetic backgrounds and in a wider range of germplasms?

One way of validating a QTL is to apply comparative mapping, i.e. to compare the results achieved in different genetic backgrounds. This is especially informative when the mapping populations have one parent in common. QTL studies on heading date and winter survival have been carried out mostly in winter \times spring and in spring barley populations. For the better understanding of the genetic control of winterhardiness it is important to study these characters in a winter barley genetic background. For this purpose comparative mapping was carried out in the *Dicktoo* \times *Plaisant* winter barley population paralleling the results with *Dicktoo* \times *Morex* (Karsai et al. 1997b). The results of comparative mapping are listed below for the two major heading date QTLs, *Ppd-H1* on chromosome 2H and *Sh2* on chromosome 5H(7). On the average, 34.2% of the markers showed polymorphism between *Dicktoo* and *Plaisant*. The highest degree of polymorphism appeared in 1H(5), where 71% of the markers tested were polymorphic. On chromosome 5H(7) this value was close to the average, 36.4%, while on chromosome 2H it was only 6%. In the vicinity of the *Ppd-H1* locus no polymorphism was found between the two winter barleys, making it evident that they carry the same allele of *Ppd-H1*. In this population significant QTLs were detected in the vicinity of *Sh2* for the frost tolerance at -14°C and for heading date under an 8-h daylength regime in the unvernallized treatment. The frost tolerance QTL contributed 26.4% to the total variance with a LOD score of 2.0, the *Dicktoo* allele increasing frost tolerance by 12.6%. The heading date QTL under the short daylength regime explained 18.8% of the phenotypic variance with a LOD score of 3.2, and lines with the *Dicktoo* allele flowered 2.2 days later. In contrast to the spring \times winter barley population, however the *Sh2* locus did not influence the heading date under long photoperiod regimes, showing that *Sh2* has a less prominent effect on adaptational traits in *Dicktoo* \times *Plaisant*. In this population one region on chromosome 1H(5) was an additional significant component of frost tolerance, explaining 25.6% of the phenotypic variance (LOD 2.4). Based on the common marker in the two mapping populations it is probable that these two winter barleys segregate at the *sh3* locus, *Dicktoo* carrying the winter growth habit allele, which increased the frost tolerance by 12.2% in the segregating population. These results emphasise that there is a major difference in the genetic determination of plant developmental rate between spring and winter barleys, but when their effect is excluded due to similarity in allele composition, different sets of chromosomal regions may become significant within each group.

To evaluate the weights of these QTL regions on adaptational traits in a wider genetic background 38 barley varieties of different geographic origin were included in association studies. The adaptational types of these varieties were determined based on their frost tolerance, vernalization response, photoperiod

sensitivity and heading dates under different photoperiod regimes (Mészáros et al., 1997; Karsai et al., 2001). Based on cluster analysis the barley varieties could be separated into seven groups. The spring barleys were placed in two, and the winter barleys in five groups representing different adaptational types. To characterise their compositions at the *Ppd-H1* and *Sh2* loci, one marker linked to each of these loci was chosen, and its correlation with the adaptational traits was determined.

In the case of photoperiod sensitivity the varieties were characterized using *CDO64*, an RFLP marker, based on the results of Pan et al. (1994). Three of the winter barleys carried the "1" allele, and all the others the "0" allele. This allele composition showed a significant correlation with heading date under 14 h in the vernalised treatment ($R^2=0.376^{**}$, Fig. 1), and with frost tolerance at -10°C ($R^2=0.442^{**}$). On the average, varieties with the "1" allele headed 17.0 days later and had a 9.3% lower survival rate. In the case of spring barleys, 9 varieties carried the "1" allele, which significantly influenced their vernalization response ($R^2=0.343^{**}$) probably through the effect of the short-day photoperiod applied during the cold treatment. The heading of these varieties was delayed by the vernalization treatment by 5.1 days, on average.

On chromosome 5H(7) *mR* (awn roughness), a morphological marker, is known to be close to the *Sh2* locus (Takahashi and Yasuda, 1970). All the 17 winter barley varieties possessed rough awns. Interestingly, 13 of the spring barley varieties carried the same allele type, and only 7 had smooth awns. This allele composition showed a correlation with heading dates in the 18-h and 14-h photoperiods in the unvernallized treatment ($R^2=0.300^*$ and 0.373^{**}), and in the 14-h photoperiod in the vernalized treatment ($R^2=0.485^{***}$, Fig. 2). Spring barley varieties possessing the winter barley allele headed significantly later in all three cases.

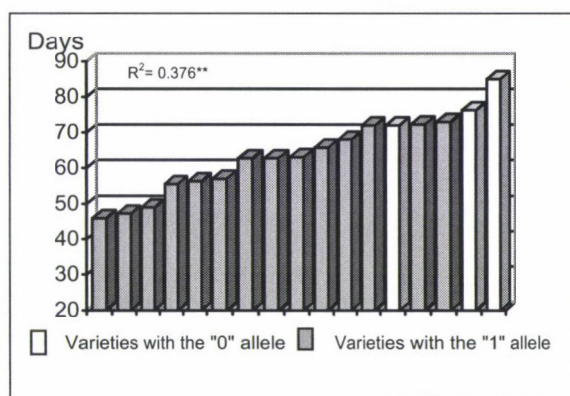


Fig. 1. Heading date of 17 winter barley varieties vernalized under a 14-hour photoperiod regime and its association with the allele phase for *CDO64*, which is linked to the *Ppd-H1* photoperiod locus on chromosome 2H

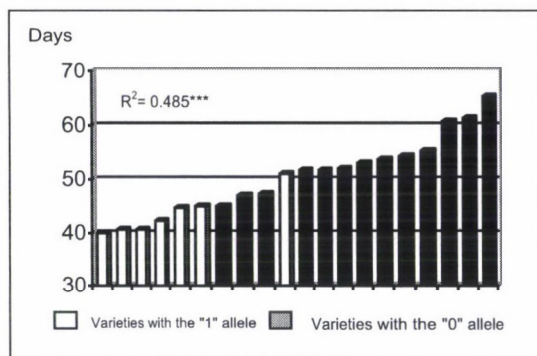


Fig. 2. Heading date of 20 spring varieties vernalized under a 14-hour photoperiod regime and its association with the allele phase for *mR*, which is linked to the *Sh2* vernalization response locus on chromosome 5H(7) (where the "0" allele is the winter barley allele)

Conclusions

The results of QTL analyses, comparative mapping and association studies give a good example of the possibility of identifying QTLs with major effect and validating QTL regions in different sets of environments and in a wider genetic background. When QTLs with major effects on a given trait appear consistently through environments and in different mapping populations, and show a significant correlation with the phenotypes in a wider germ plasm, this proves that they are real genetic determinants of the given trait. This knowledge makes it possible to continue these experiments in the direction of marker-assisted selection and/or gene isolation through marker saturation of the relevant chromosomal regions and map-based cloning. The latter can give an insight into the exact mechanism through which the gene determines the phenotype.

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PLANT GAMETES AS TOOLS FOR MOLECULAR BREEDING

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Sexual reproduction plays an essential role in the propagation of Angiosperms. Fertilisation takes place in the embryo sac, which is usually deeply encased in the sporophytic tissues of the ovule. In contrast to animals and primitive plants, the mechanism of egg cell activation in flowering plants has not been discovered fully because of the inaccessibility and complexity of the process of double fertilisation. However, recent advances in plant cell and molecular biology have brought new, powerful technologies to investigate and micromanipulate the reproductive cells of flowering plants including cereal crops.

An experimental approach based on various micromanipulation techniques involving *in vitro* fertilisation (IVF) and microinjection procedures is now available in more and more laboratories. Despite some limitations this offers new possibilities to study cellular and subcellular events preceding or occurring during or after egg cell activation and early embryonic development. Recent achievements in the field of wheat egg cell micromanipulation are presented in this paper.

Key words: wheat, egg cell, micromanipulation, microinjection, IVF

Introduction

Till now much of our basic knowledge on the sexual reproduction of flowering plants has been restricted to comparative and descriptive information obtained from morphological, histological, cytological and physiological studies. As the *in vitro* fertilisation technique using isolated male and female gametes was improved (Kranz et al., 1991; Faure et al., 1994; Kovács et al., 1995) and zygotes produced *in vitro* could be regenerated into fertile plants (Kranz and Lörz, 1993) the examination of the intimate mechanism of double fertilisation became possible not only at the cellular, but also at the molecular level.

However, there is a considerable time lag in the field of plant embryology as compared to animal and human embryology, mainly due to the fact that the female gametophyte of angiosperms is inaccessible for direct micromanipulation. Recent advances in plant cell and molecular biology have brought new, sophisticated experimental techniques to investigate the reproductive cells of flowering plants (including cereal crops) and to micromanipulate them for various biotechnological purposes (Dresselhaus et al., 1996; Leduc et al., 1996; Kumlehn et al., 1998; 1999; Pónya et al., 1999a; Holm et al., 2000). Thus, subcellular events preceding fertilisation and occurring in the course of *in vitro* gametic fusion can be approached, together with a number of other important questions such as how and when cell polarity is established in the egg cell, what factors trigger the first asymmetric division of the zygote and what sorts of genes govern these mechanisms.

Some of these questions can only be addressed if systems of gamete isolation and fusion are associated with the delivery of foreign DNA or various fluorescent dyes into the gametes/zygotes of higher plants.

When it comes to attempting to introduce foreign DNA into the germ cells (the number of which is severely limited), the method of choice may be microinjection, which allows the targeting of cellular compartments (especially the nuclear region) more easily than other transformation techniques (Crossway et al., 1986). However, the problem of cell immobilisation has to be circumvented if the microinjection of DNA or other macromolecules into single plant cells/protoplasts is to be achieved.

As an agronomically important crop, wheat has been used for a set of experiments aimed at carrying out various micromanipulations at the gametic level.

Isolation of viable male and female gametes in wheat (*T. aestivum* L.)

The application of micromanipulation techniques makes the isolation and *in vitro* fusion of female and male angiosperm gametes possible (Kranz and Kumlehn, 1999).

Only a very limited number of publications (Matthys-Rochon et al., 1987; Szakács and Barnabás, 1989; Kovács et al., 1995) have dealt with sperm cell isolation from wheat pollen. This is probably due to the fact that wheat pollen grains are short-lived (up to 30 min) after shedding, which makes the isolation procedure difficult. Viable wheat sperm cells were isolated in large numbers in our laboratory by the application of osmotic shock and gentle agitation in BK medium (Brewbaker and Kwack, 1963) containing 20% sucrose or sorbitol with pH 5.8 (Figs.1–2) (Szakács and Barnabás, 1989). After filtration and density gradient centrifugation the sperm cells kept their original shape and showed intensive fluorescence by fluorescein diacetate in BK medium containing 20% sucrose or sorbitol. Viability was retained until the end of the procedure and for a further 15 min (approx. 30 min in all) at room temperature (20°C). After isolation, sperm cells could be individually selected under the microscope and then transferred into fusion droplets to complete IVF.

The isolation of viable egg cells of wheat was achieved by applying microsurgery without enzymatic maceration of the ovules (Kovács et al., 1994). Under a binocular microscope ovules were dissected from ovaries collected 7 to 14 days after emasculation. Pieces of ovules from the micropyle region were put in plastic microdishes filled with isolation medium (630 mosmol mannitol/l H₂O, pH 6.0). Egg cells were isolated from the female sporophytic tissues with fine glass needles under an inverse microscope. The isolated egg cells were viable even 2 h after isolation. The mean isolation frequency was 20% or more using this procedure.

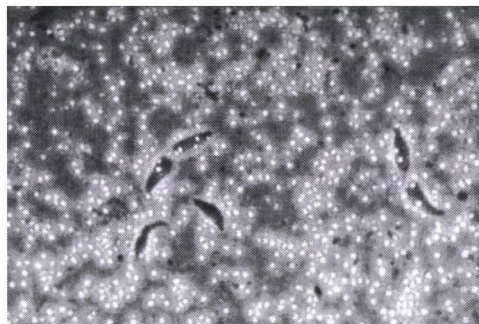


Fig. 1. Sperm cells released from a pollen grain by osmotic shock (phase contrast microscopy)



Fig. 2. Pair of isolated sperm cells of wheat (phase contrast microscopy)

Special morphological features of wheat egg cell maturation

If manipulations of gametophytic cells are to be successful they must be carried out in the context of a comprehensive knowledge of structure and function and at the correct phase of plant ontogeny.

Our work on wheat (*Triticum aestivum* L.) represents the commencement of a series of investigations on the functionality of the female gametophyte and on its suitability for various micromanipulations.

Cultivated wheat is a protogynous plant in which the Polygonum-type embryo sac develops 3–4 days before anthesis (Tímár et al., 1997). Biological studies carried out on the flowering of higher plants in the 60s in connection with the development of hybrid wheat (Rajki-Cicer, 1961) had already indicated the long-lasting receptivity of the wheat pistil. Detailed observations were made on egg cell protoplasts isolated mechanically from pistils removed from the spring wheat cultivar Chinese Spring at various stages and then fixed in agarose. After fixation and the embedding procedure, semi- and ultrathin sections were cut from the protoplasts for light and transmission electron microscopic studies. Examinations of the cell structure of wheat egg cell protoplasts (Pónya et al., 1998b) isolated from young (3 days prior to anthesis) and over-aged (12 days after anthesis) caryopses confirmed that wheat caryopses had a long life-span. The nucleus of the egg cell, which became spherical during the isolation (Fig. 3) was centrally located. The characteristic cell organelles (mitochondria, amyloplasts, lipid bodies) surrounded the nucleus, while the numerous, relatively large vacuoles were in a peripheral position.

The maturation of the egg cell was accompanied by a number of fundamental morphological changes, one of which was the large increase in size. Six days after anthesis the initial volume of the egg cell doubled, after which it gradually decreased in volume during aging. The changes in the volume of the protoplasts were reflected in an increase in the size of the nuclei and the nucleoli. The aging of the egg cell was accompanied by the degradation of the chromatin reserves of the nucleus, while the accumulation of reserved nutrients could be observed in the cytoplasm. There was a decline in the degree of vacuolisation, with the vacuoles becoming smaller and more uniformly distributed. In egg cells isolated 2 weeks after anthesis the characteristic symptoms of programmed cell death (apoptosis) could be observed (Pónya et al., 1999a; Tímár et al., 2002).

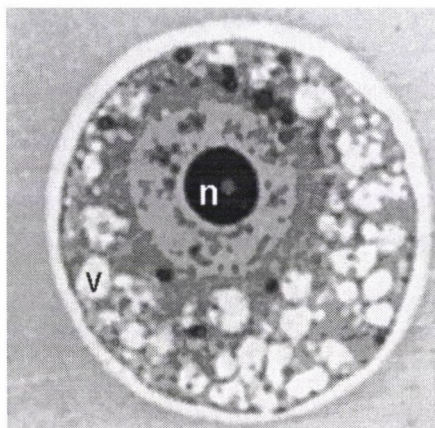


Fig. 3. Transmission electron micrograph of a wheat egg cell isolated 1 day before anthesis (n: nucleus; v: vacuoles)

***In vitro* fertilisation and zygote culture of wheat**

The successful *in vitro* fertilisation and zygote culture of angiosperms is the most important breakthrough in experimental research on sexual plant reproduction during recent years (Kranz and Dresselhaus, 1996). Tissue culture methods which are adapted for the culture of single somatic or haploid cells allow the individual development of zygotes developed both *in planta* and *in vitro* into fertile plants. The IVF system can be a useful model to study embryo development (Kranz and Kumlehn, 1999).

As a result of collaborative work between German and Hungarian researchers the electrofusion-mediated fertilisation of single egg cells of wheat with isolated and individually selected sperm cells was successfully carried out for the first time (Kovács et al., 1995). The fusion frequency varied between 30–55%. Two days after fusion 60% of the artificial zygotes started to divide and more than 80% of them formed multicellular structures (including microcalli) in a somatic feeder cell culture of maize. With the use of embryogenic barley or wheat microspore cultures fertile wheat plants could be regenerated from zygotes cultured *in vitro* (Kumlehn et al., 1999; Bakos et al., unpublished data).

A novel method of immobilising and microinjecting egg cells of wheat

A highly efficient technique of microinjection has been elaborated in our laboratory. Instead of generally used methods (embedding protoplasts in agarose droplets or stabilising them using a holding pipette) freshly isolated egg protoplasts were exposed to a high frequency alternating current (AC) field for immobilisation (Pónya et al., 1999a). The technical parameters (injection pressure, volume of the aliquots which could be introduced into the protoplasts without causing bursting, preparation of microneedles, etc.) had to be optimised before the successful microinjection of various macromolecules into isolated egg cells could be achieved.

Capitalising on this novel immobilisation method, the microinjection of fluorescent phalloidin into wheat egg cells made it possible to follow the *in vivo* dynamics of the F-actin cytoskeleton (Pónya and Barnabás, 2001). The fluorophore bound to the actin microfilaments within minutes following microinjection. The localisation of F-actin was investigated in egg cells of wheat developed *in situ* and fertilised *in vitro* at consecutive time intervals using a low-light CCD camera connected to an image-processing system. The images of filamentous actin revealed the rapid, dynamic reorganisation of the actin filaments upon sperm-egg cell fusion. Whereas unfertilised egg cells showed the polar F-actin localisation, which was most pronounced in receptive egg cells, cortical actin filaments were found to be distributed uniformly in egg cells fertilised *in vitro* after the characteristic accumulation of an actin patch at the site of sperm entry had disassembled. Throughout mitosis and cytokinesis, the rearrangement of the interphase actin cytoskeleton resulted in transverse cortical F-actin becoming concentrated in a widening band predicting the future division plane. These studies may provide insight into the role of F-actin in regulating the preparation of the egg cell for fertilisation and into fertilisation-related events.

Experiments on the microinjection of foreign DNA into egg cells immobilised on the surface of an electrode have just begun. It was observed that 8–12 picolitres of external DNA could be injected into the cell without causing injury. Through the use of constitutive promoters such as CaMV35S and maize ubiquitin, together with GFP as a reporter system, a high frequency (up to 74%) of transient gene expression could be detected (Pónya et al., 1999a) in the injected wheat egg cell protoplasts.

The microinjection procedure coupled with the electrofusion system, makes it possible to attempt to transform cereals using the sexual route and to produce transgenic plants from single cell cultures.

Egg cell activation in flowering plants

The discovery of the entire mechanism of egg cell activation is now an exciting area of developmental biology. The potential similarities/dissimilarities between egg activation in animals and in higher plants are gradually being unravelled (Antoine et al., 2001).

Unpublished results (Pónya and Barnabás, unpublished data) demonstrate that, as in the case of animals, the release of calcium from internal stores is the primary cause of egg activation. The endoplasmic reticulum (ER), which is a key organelle involved in various metabolic processes of plant cells, might serve as the main calcium store in wheat egg cells. IVF systems permit the further analysis of fusion and activation events without the influence of maternal tissues.

All the research achievements presented here might contribute to the utilisation of plant sexual cells in the field of molecular breeding.

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MOLECULAR CYTOGENETIC ANALYSIS OF WHEAT-ALIEN HYBRIDS AND DERIVATIVES

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New wheat × barley, wheat × *Aegilops biuncialis* and wheat × rye hybrids were produced with the aim of alien gene transfer from these species into wheat. Amphiploids were produced with the help of colchicine treatment from the last two combinations. The new wheat × barley hybrids were multiplied in tissue culture because of the high degree of sterility and then pollinated with wheat to obtain backcross progenies. Wheat-barley chromosome pairing was detected using genomic *in situ* hybridization (GISH) in two combinations (Mv9 kr1 × Igri, Asakazekomugi × Manas). *In vitro* conditions caused an increase in chromosome arm association frequency in both combinations and in fertility in some regenerants.

Five wheat-barley translocations were produced in a wheat background and characterized through the combination of cytogenetic and molecular genetic approaches (GISH, FISH and SSR markers). The following translocations were identified: 2DS.2DL-1HS, 3HS.3BL, 6BS.6BL-4HL, 4D-5HS and 7DL.7DS-5HS. Physical mapping of the SSR markers on chromosomes 1H and 5H was carried out using the intragenomic and interspecific translocation breakpoints and the centromere as physical landmarks.

Disomic wheat-*Aegilops biuncialis* additions were produced after backcrossing the wheat-*Ae. biuncialis* amphiploids. Fluorescence *in situ* hybridization (FISH) was carried out using two repetitive DNA clones (pSc119.2 and pAs1) on *Ae. biuncialis* and its two diploid progenitor species to detect chromosome polymorphism. The 7M and 3M disomic chromosome additions were selected and five more lines still need to be characterized.

The octoploid triticale (Mv9 kr1 × Lovászpatonai) produced in Martonvásár was crossed with a 1RS.1BL wheat cultivar Matador. GISH analysis detected pairing between the 1RS arm of the translocation chromosome and that of Lovászpatonai rye in 32 % of the pollen mother cells, making it possible to select recombinants from this combination. The new recombinants between the 1RS of Petkus and the 1RS of Lovászpatonai rye cultivars are being analysed with the help of microsatellite markers.

Key words: wheat, barley, rye, *Aegilops biuncialis*, intergeneric hybrids, *in situ* hybridization

Introduction

Species related to wheat have great genetic diversity for the majority of characters. By means of interspecific and intergeneric crosses with the *Triticeae* many favourable traits can be transferred to wheat (Belea, 1986; Friebe et al., 1996). The homoeology between wheat and its related species makes it possible for alien chromosome segments to compensate for the missing wheat chromosome, as well as introducing new, favourable genes.

In situ hybridization (ISH) is a powerful method to localize nucleic acid sequences (either DNA or RNA) in the cytoplasm, organelles, chromosomes or

nuclei of biological material. One of the important modifications of the ISH technique is genomic *in situ* hybridization (GISH) (Le et al., 1989; Schwarzacher et al., 1989), which utilizes total genomic DNA from one of the parental species as a probe and allows chromosomes of different parental origins to be "painted" in different colours in the nuclei of interspecific hybrids. Fluorescence *in situ* hybridization (FISH) using repetitive DNA probes gives a specific chromosome pattern, which makes this method suitable for chromosome identification in the tribe *Triticeae* (Mukai et al., 1993; Rayburn and Gill, 1985; 1986).

In the present experiments the aim was to develop new winter wheat \times winter barley, winter wheat \times *Aegilops biuncialis* and winter wheat \times rye hybrids, addition and translocation lines. It is planned to transfer the earliness and favourable protein composition of winter barley (*Hordeum vulgare*), the good salt and drought tolerance of *A. biuncialis* and the rust resistance of rye (*Secale cereale*) into wheat. The identification of the chromosomes in interspecific hybrids and their derivatives was carried out using molecular cytogenetic methods. GISH was used to differentiate the chromosomes of different species in the intergeneric hybrids and in their derivatives. Sequential GISH and FISH with two repetitive probes was used for the identification of the chromosomes in the alien translocation lines.

Wheat (*Triticum aestivum*) – barley (*Hordeum vulgare*) hybridization

Bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are two of the most important cereal crops worldwide. Hybridization between these species makes it possible to transfer desirable traits (e.g. earliness) from barley into wheat. The prerequisite for the successful transfer of chromosome segments is pairing between the chromosomes of the different species in the hybrids, which may result in the development of recombinants. The first successful hybridization between wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) was reported by Kruse (1973) and not much later a set of wheat-barley addition lines was produced (Islam et al., 1978). The addition lines were produced from the Chinese Spring wheat and Betzes barley varieties, which have high crossability in intergeneric crosses but are unsatisfactory from the agronomic point of view. For the successful transfer of useful genes from barley into wheat it is essential to produce hybrids with genotypes which carry agronomically important characters. However, very few hybrid combinations have been reported from wheat \times barley crosses involving barley varieties with good agronomic characters (Wojciechowska and Pudelska, 1993; Jauhar, 1995; Taketa et al., 1998) and in most cases no backcross progenies were developed.

Fedak (1977), Sethi et al. (1986) and Jauhar (1995) assumed that homoeologous pairing occurred between the chromosomes of wheat and barley on the basis of the chromosome pairing frequency data observed in the hybrids. Islam and Shepherd (1988) observed pairing between barley telocentrics and wheat

chromosomes at a frequency of 1.2–4.5%. Wheat-barley chromosome pairing can result in the development of recombinants between the chromosomes of these species. Up till now only a few wheat-barley translocations have been developed (Islam and Shepherd, 1992; Koba et al., 1997; Endo et al., 1998; Sherman et al., 2001). In Martonvásár wheat-barley translocation lines were produced from hybrids regenerated from tissue culture (Molnár-Láng et al., 2000a).

The main objectives of the experiments were to produce new wheat-barley hybrids and addition lines between agronomically useful genotypes, and to detect wheat-barley chromosome pairing in meiosis in the hybrids with the aim of assessing the frequency of wheat-barley recombinants and of analysing the translocations produced in Martonvásár using cytogenetic and molecular genetic methods.

Wheat-barley chromosome pairing detected by GISH in the new winter wheat × winter barley hybrids produced in Martonvásár

Fourteen winter barley and two spring barley cultivars were tested for use as pollinators in wheat (*Triticum aestivum* L.) × barley (*Hordeum vulgare* L.) hybrid production and hybrids were successfully produced with three different barley genotypes: Igri (German two-rowed winter barley variety), Osnova and Manas (Ukrainian six-rowed winter barley varieties) (Molnár-Láng et al., 2000b). Mv9 kr1 × Igri, Mv9 kr1 × Osnova and Asakazekomugi × Manas hybrids were produced besides the Chinese Spring × Betzes hybrid created earlier (Molnár-Láng and Sutka, 1994). The seed set was extremely low even in the successful combinations. No seed set was observed when wheat was pollinated with the other thirteen barley varieties tested.

The new winter wheat × winter barley hybrids were multiplied in tissue culture because of the high degree of sterility and then pollinated with wheat to obtain backcross progenies. When meiotic analysis of the hybrids Mv9 kr1 × Igri and Asakazekomugi × Manas and of progenies regenerated *in vitro* was carried out with the Feulgen method 1.59 chromosome arm associations per cell were revealed in both initial hybrids. The number of chromosome arm associations increased after *in vitro* culture to 4.72 and 2.67, respectively, in the two combinations. GISH was carried out according to Reader et al. (1994). According to the GISH analysis wheat-barley chromosome arm associations made up 3.6% of the total in the initial Mv9 kr1 × Igri hybrid and 6.6% and 16.5% of the total in progenies of the Asakazekomugi × Manas and Mv9 kr1 × Igri hybrids, respectively, regenerated *in vitro*. The demonstration by GISH of wheat-barley chromosome pairing in the hybrids and especially in progenies regenerated *in vitro* proves the possibility of producing recombinants between these two genera, and thus of transferring useful characters from barley into wheat. *In vitro* conditions caused an increase in chromosome arm association frequency in both combinations and in fertility in some regenerants (Molnár-Láng et al., 2000b).

Production of winter wheat cv. Mv9 kr1/winter barley cv. Igri addition lines and identification of the barley chromosomes using GISH

Mv9 kr1 \times Igri hybrid regenerants multiplied *in vitro* were backcrossed with the wheat line Mv9 kr1; after pollinating 4606 flowers with wheat, nine embryos could be dissected, and six BC₁ plants were grown to maturity (Molnár-Láng et al., 2000b). The BC₁ plants were backcrossed again with the wheat line Mv9 kr1 and 24 BC₂ seeds were obtained, 16 of which produced self-fertile plants. The chromosome numbers of the BC₂ plants ranged between 43 and 46. On the BC₂ plants 735 seeds developed after self-pollination. Plants with 43 and 44 chromosomes were selected from the selfed BC₂ seeds after Feulgen chromosome counting. Meiotic analysis will be employed to decide whether the plants with 44 chromosomes carry an additional pair of barley chromosomes or two different ones. Up till now no plants have been identified with 22 bivalents showing up as disomic additions; however, the meiotic pairing behaviour of only 12 of the 24 plants with 44 chromosomes has so far been analysed.

Wheat \times maize crosses are also used to produce disomic additions from monosomic additions. When twenty plants with 43 chromosomes were pollinated with maize (cv. Seneca), it was possible to dissect 22 embryos. Fourteen embryos started to germinate and 6 plantlets developed. The six plants are now being grown in a growth chamber and their chromosome numbers will be checked later on.

The expected transmission ratio of the alien chromosome from the monosomic plants is higher through the female gametes than through the male gamete. It is hoped that different additions can be recovered after colchicine treatment on haploid plants carrying one alien chromosome in the wheat background.

Production and identification of wheat-barley translocations using GISH, FISH and SSR markers in the progenies of wheat-barley hybrids multiplied in tissue culture

Wheat-barley translocations were identified by genomic *in situ* hybridization (GISH) in backcross progenies originating from wheat \times barley (Chinese Spring \times Betzes, Mv9 kr1 \times Igri) hybrids regenerated *in vitro*. The regenerated hybrids were pollinated with the wheat line Martonvásári 9 kr1. All were single breakpoint translocations with the relative positions of the breakpoints ranging from the centromere to about 0.8 of the relative arm length (Molnár-Láng et al., 2000a).

Five wheat-barley translocations in a wheat background were characterized through the combination of cytogenetic and molecular genetic approaches. The wheat chromosome segments involved in the translocations were identified using sequential GISH and two-colour FISH with the DNA probes pSc119.2 and pAs1. The barley chromatin in these lines was identified using SSR markers. A total of 45 markers distributed over the total barley

genome were selected from a recently published linkage map of barley (Ramsay et al., 2000) and screened on the translocation lines. The following translocations were identified: 2DS.2DL-1HS, 3HS.3BL, 6BS.6BL-4HL, 4D-5HS and 7DL.7DS-5HS (D. Nagy et al., 2002). Wheat-barley disomic and ditelosomic addition lines for the chromosomes 3HS, 4H, 4HL, 5H, 5HL and 6HS were used to determine the correct localization of 21 markers and the position of the centromere. An ancient intragenomic rearrangement between chromosome arms 1HL and 5HS was detected in barley. Physical mapping of the SSR markers on chromosomes 1H and 5H was carried out using the intragenomic and interspecific translocation breakpoints and the centromere as physical landmarks.

Four of the five translocation lines have good fertility and are being multiplied in the nursery. The translocation lines show differences in a number of morphological traits, such as ear type, the length of the ear, awns, plant height, earliness, etc. The agronomic traits of these lines will be studied in more detail when more seeds are available.

These lines could significantly improve the accuracy with which markers and genes can be assigned to chromosome regions in the barley genome. Furthermore, the translocation lines will contribute to extending the genepool available for wheat improvement.

Production and molecular cytogenetic identification of *Triticum aestivum*/*Aegilops biuncialis* disomic addition lines

Aegilops biuncialis Vis. ($2n=4x=28$, UUMM) is a tetraploid wild relative of wheat. The U genome progenitor is the diploid *Ae. umbellulata* Zhuk. ($2n=2x=14$, UU), while the modified M genome originated from *Ae. comosa* Sm. in Sibth. & Sm. ($2n=2x=14$, MM) (Kimber and Sears, 1983). *Ae. biuncialis* represents a promising source of genes for resistance to barley yellow dwarf luteovirus, yellow rust and brown rust that could be used for wheat improvement. Direct gene transfer could be carried out by producing alien chromosome additions in bread wheat.

The aim was to detect chromosome polymorphism between *Ae. biuncialis* and its diploid progenitors and to identify the disomic *Ae. biuncialis* chromosome additions produced in Martonvásár in a winter wheat background using FISH with two repetitive DNA clones, pSc119.2 and pAs1. The FISH karyotype determined with these two probes for the diploid *Aegilops* species was reported by Badaeva et al. (1996), and that of the tetraploid *Ae. cylindrica* by Linc et al. (1999), but no paper has yet reported the FISH hybridization pattern of the tetraploid *Ae. biuncialis*.

Most of the *Ae. biuncialis* chromosomes showed a hybridization pattern similar to that of the diploid progenitor species, but chromosome polymorphism was detected on some chromosomes after FISH. *Ae. biuncialis* was crossed as

male parent with the winter wheat line Martonvásári 9 kr1 and F₁ hybrids were produced. These were treated with colchicine and the amphiploid plants obtained were backcrossed with wheat (Logojan and Molnár-Láng, 2000). Six different lines with 44 chromosomes were selected. These showed 22 bivalents in metaphase I in meiosis, demonstrating that they were disomic additions of *Ae. biuncialis* in the wheat genome. FISH was carried out on mitotic chromosome preparations of these lines using the DNA clones pSc119.2 (labelled with fluorogreen) and pAs1 (labelled with fluorored) to identify the *Ae. biuncialis* chromosomes.

Line No. 7753 carried an extra 3M chromosome pair originating from *Ae. biuncialis*. The 3M chromosome had a specific but very faint doubled pAs1 hybridization site on both its arms in a terminal position. There was no pSc119.2 hybridization site on the 3M chromosome.

Line No. 6311 was identified as a 7M disomic chromosome addition. Besides the wheat chromosomes the two *Ae. biuncialis* chromosomes were identified as 7M. This 7M chromosome had a specific doubled pAs1 site on the short arm terminally and on the long arm as well, and carried a strong subterminal pSc119.2 hybridization site on the long arm.

Lines No. 8015 and No. 4951 had a small submetacentric *Ae. biuncialis* chromosome pair, which will require further molecular cytogenetic analysis, as will the other lines with 44 chromosomes (Linc et al., 2002).

Production and molecular genetic identification of new wheat-rye recombinants

The short arm of chromosome 1R of rye (*Secale cereale* L.) carries several genes of agronomic importance, which have been transferred into wheat (*Triticum aestivum* L.) in the form of different translocations occurring between 1R and its wheat homoeologues (for review see Friebe et al., 1996; Koebner and Shepherd, 1986). The most widespread of these is a 1B/1R translocation, in which the short arm of the 1B chromosome is replaced by that of 1R from rye cultivar Petkus. This translocation occurred spontaneously in a Crieewener 104 × Petkus hybrid produced in the 1930s (Schlegel and Korzun, 1997). It has improved resistance to several foliar diseases such as stem rust (*Puccinia graminis* Pers.), leaf rust (*Puccinia recondita* Robillard ex Desm.), yellow rust (*Puccinia striiformis* West.) and powdery mildew (*Erysiphe graminis* De Candolle ex Merat) since it bears the Sr31, Lr26, Yr9 and Pm8 resistance genes, respectively (Zeller, 1973; Bartos and Bares, 1971; Bartos et al., 1973), as well as having a positive effect on the productivity of its carriers (Rajaram et al., 1983). However, it confers undesirable intense dough stickiness (Dhaliwal et al., 1988), in addition to which the resistance against some of the above-mentioned diseases has now been broken down in most 1RS.1BL cultivars (Bedő et al., 1993).

It was planned to introduce 1RS chromosome arms carrying agronomically valuable genes different from those of Petkus into wheat varieties by means of homologous recombination between the original 1RS.1BL translocation and the 1RS arm of some other selected rye genotypes.

When the wheat line Martonvásári 9 kr1 was pollinated with the rye cultivar Lovászpatonai the seed set was as high as 68.4 % (D. Nagy et al., 1998). The F₁ hybrid plants were treated with colchicine and up to 38 amphiploid seeds were obtained after selfing. The rye chromosomes in the amphiploids were detected with genomic *in situ* hybridization using total rye DNA as a probe. The octoploid triticales plants were crossed with the 1RS.1BL wheat cultivar Matador. The frequency of chromosome associations involving the 1RS.1BL translocation was examined at metaphase I of meiosis in BC₁ plants using GISH. Most of the chromosome associations were rod bivalents (67%) and trivalents (26.6%); one quadrivalent was observed (1.2%). The 1RS arm of the translocation chromosome paired with that of Lovászpatonai rye in only 32% of the pollen mother cells, whereas the translocated 1BL arm exhibited much higher pairing (90%) with its wheat homo(oe)logues (D. Nagy and Molnár-Láng, 2000).

When the secalin patterns of the Martonvásári 9 kr1 × Lovászpatonai octoploid triticales and the 1RS.1BL wheat cultivar Matador were analysed, some octoploid triticales lines showed a different secalin pattern from the original 1RS.1BL translocation. Recombinants were selected from selfed progenies of the (Martonvásári 9 kr1 × Lovászpatonai) × Matador combination. The presence of the 1RS.1BL translocation was detected using GISH and recombinants were selected according to the secalin pattern. At present the new wheat-rye recombinants are being analysed with rye microsatellite markers.

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SEED PRODUCTION ASPECTS OF GENETICALLY MODIFIED CROP VARIETIES

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The seed multiplication of genetically modified (GM) plants requires a modification of the multiplication process used for conventional seed. The difference compared to conventionally-bred varieties involves the detection of the modified character during variety maintenance, seed multiplication and processing, the need for separate storage, processing and transportation, the extra cleaning required for the transportation, storage and processing equipment, and the extra administration necessary for the documentation and labelling of GM seed lots. All in all this results in the lower exploitation of seed-producing capacity and in additional costs.

The appearance of GM plants also has an effect on the breeders and seed multipliers of conventional varieties, however, since the possibility of contamination cannot be excluded. The producers of seed free of GMs (within the tolerance threshold) are forced to employ costly cultivation techniques (temporal and spatial isolation, removal of volunteer plants) and laboratory tests (for GM contamination) if their varieties are to stay on the market and if it is to remain possible to produce GM-free products in the future.

Key words: maintenance breeding, seed production, genetically modified plants, contamination

Introduction

For many decades now it has been natural in the majority of countries that only certified, standard seed of state registered varieties can be marketed. State variety trials provide considerable protection to growers and breeders alike. Traditionally, the state registration of economically important plant species is preceded by several years of experimentation in the course of which the variety must prove that it is capable of yielding at least as much, if not more, than the control varieties, and that it can be cultivated with no less risk than earlier varieties since it has a satisfactory level of frost resistance, straw strength, disease resistance, etc., properties that are important for yield stability. The introduction of state variety registration is also advantageous for breeders, since, although the sanctions vary from country to country, it provides protection against the unauthorised use of varieties and thus ensures that the capital invested in plant breeding brings satisfactory returns.

Growers wish to cultivate varieties whose yield potential and yield stability allow them to gain a satisfactory income, while their quality traits conform with consumer demands. One aim of seed production is to produce variety-pure seed which is genetically identical to the plant population with known characters tested in the official trials. The other aim of seed production is to ensure that the seed quality (germination ability, vigour, purity, etc.) satisfies

cultivation requirements, allowing the optimum plant density for the plant species to be achieved. Seed production regulations and seed quality inspections must be designed to achieve these two aims. In all countries where agriculture is carried out at a high standard, the parameters of variety identity and seed quality are laid down in standards, compliance with which is obligatory. The breeding of GM plant varieties demands a rethinking and modification of the variety registration and seed certification system which has functioned well for many decades. If the results of biotechnological research are to be put into practice in the form of cultivated GM varieties, seed production must conform to special criteria in addition to the classical regulations.

Legal regulation of seed production

In all countries seed production, processing and marketing are subject to strict state control. Although the legal details may deviate and certain national differences can be observed, the laws follow the same principles. The laws in force in various developed countries specify that

- only the seed of state-registered plant varieties or those with special permits (for export purposes, advanced lines) can be multiplied;
- only seed which satisfies the quality criteria can be used and marketed for multiplication or commodity production;
- seed quality criteria are laid down in standards and the quality is inspected by state officials;
- the seed of legally protected varieties can only be multiplied and marketed with the permission of the variety owner;
- special restrictions are in force in various countries regarding the field location, transportation, storage, labelling, etc. of GM plants intended for cultivation or multiplication.

The initial stock for the production of plant seeds consists of breeder's seed, i.e. variety-identical seed lots produced each year by the breeder or variety maintainer. If sufficient seed is to be available for commodity production, this initial stock needs to be multiplied over the course of several years, generally 3 or 4 years, depending on the plant species and its multiplication rate.

Requirements to be met by the seed

Variety identity, homogeneity

One precondition for variety registration is that the variety should conform to the UPOV (Union Internationale pour des Obtentions Végétales – International Union for the Protection of New Varieties of Plants) and DUS (distinctness, uniformity, stability) requirements. This ensures that the plant varieties are morphologically distinct from one another, while the individual plants within the variety population are uniform and stable, i.e. they remain unchanged from year to year. The UPOV directives stipulate in detail the

homogeneity requirements to be met by major plant species. In the case of cereals, for example, the homogeneity of over 30 obligatory or recommended morphological traits must be tested on the variety population and on 100 lines each. The plant variety can be regarded as homogeneous if at most three of the approx. 3000 morphological data recorded for the 100 lines differ from those laid down in the variety description.

Variety maintenance is carried out to ensure that the homogeneity of the variety is maintained. Each year positive selection is carried out for variety-identical plants and those which differ from the variety description are removed from the basic stock. Plants which differ morphologically from the variety may appear in the population for various reasons. In the case of self-pollinating plants and the parental lines of hybrid plants a low extent of segregation may occur even after the F_{10} generation if the variety is not 100% homozygous, leading to the appearance of morphologically differing plants (Allard, 1960). Spontaneous mutations also occur with a frequency of 10^{-5} – 10^{-7} , some of which will be manifested as a change in morphological traits (Bálint, 1980). Depending on the environmental conditions varying extents of cross pollination (biological mixing) may also endanger the maintenance of the variety in unchanged form, as may mechanical mixing. In the case of open-pollinated (non-hybrid) plant varieties, in addition to the above, it is important to ensure that the gene frequency of the progeny population is identical to that of the initial population (Bálint, 1980).

If a variety has special resistance, chemical quality or phytophysiological characters (in addition to numerous conventionally bred varieties, virtually all GM varieties fall into this category) the presence of this special trait must be checked in the lines in the course of variety maintenance. These checks are fairly simple if the trait in question is linked to morphological markers or if the variety has herbicide tolerance, but for the majority of quality traits the use of biochemical or molecular markers is necessary.

Field multiplication

The small quantity of seed originating from variety maintenance must be multiplied in such a way that the plants in the population remain identical with the variety, while the seed quality satisfies the standard requirements. Seed multiplication should be carried out under environmental conditions optimum for the given plant species, employing production technologies of a high standard. Seed-producing fields should be flat, with uniform soil and balanced nutrient supplies, so that the stand will develop uniformly. The plant density should not be too high, as the individual plants cannot be observed clearly in a dense stand and there is also a greater danger of lodging and disease. In addition, the thousand grain mass will be lower, leading to a decrease in the ratio of grains of a size suitable for use as seed.

Depending on the plant species, the forecrop on the seed-producing field for the last one (cereals) to five (sunflower, rape) years must not be the same or in some cases a related species in order to avoid mechanical or biological mixing due to voluntary emergence. This restriction does not exclude the risk of variety mixing entirely, since the seeds of most plant species are able to remain viable in the soil for a number of years, but in the case of traditional plant varieties it reduces the likelihood of mixing to an acceptable level. If conventional and genetically modified plant varieties are multiplied in the same district, other problems involved in forecrop restrictions may also arise. If a low extent of voluntary emergence occurs in the GM stand, this will have little effect on the seed multiplication, but if the same quantity of GM plants occurs in a conventional variety, this will cause considerable difficulties for the seed multiplier, who wishes to produce guaranteed GM-free seed. According to a draft directive issued by the Commission of the European Community, fields used to grow seed of non-genetically modified varieties should be free from seed of genetically modified plants from previous cropping which are still capable of germinating. According to the draft, the GM forecrop restriction is 5 years for fodder, oil and fibre plants and 2 years for other crops. The opinion published by the Scientific Committee on Plants (Opinion adopted by the Committee on 7 March 2001) concerning the adventitious presence of GM seeds in conventional seeds states that this variety grouping is too general and is based not on evidence but on experience.

In order to avoid cross pollination, the seed-producing field must also be isolated in space from other seed-producing or commodity-producing fields of the same plant species. The isolation distance depends on the type of fertilisation and flowering. In the case of self-fertilised cereals, for instance, an isolation distance of 2 m is sufficient, while seed-producing fields of maize should be at least 200–400 m from other maize fields (MSZ 6353, 1998). Due to the appearance of genetically modified plants the draft EU directive stipulates a doubling of the isolation distance in the case of open pollinated crops when non-GM hybrid plants are grown for seed. Neither the currently applied nor the recommended isolation distance is able to eliminate the risk of cross pollination completely. Even in the case of cereals, which are strictly self-pollinated, 1–5% cross pollination can be observed, and this may increase as a consequence of certain environmental conditions such as drought or heat (Tsunewaki, 1969). Considerable differences can be observed between the varieties with regard to the degree of open flowering and spontaneous male sterility (Luzatto, 1930; Nikolaeva, 1947). When the anthers are completely dehiscent they still contain sufficient pollen for cross pollination. A considerable proportion of the pollen grains are dispersed in the air and are capable of cross fertilisation. The number of pollen grains present in wheat anthers is far lower than that in the anthers of rye or maize, where fertilisation is carried out by air-borne pollen. Observations made by numerous scientists (De Vries, 1971; D'Souza, 1970; Rajki and Rajki,

1966) suggest that there may be 1000–4000 pollen grains in each anther in various wheat varieties. Only a small proportion (approx. 20%) of the pollen grains in the anthers take part in self-pollination. The remainder enter the air and are capable of pollinating other flowers. As wheat is a hexaploid, the pollen grains are larger and heavier than those of diploid and tetraploid *Triticum* species and thus fall to the ground relatively quickly (Petrovskaya-Baranova, 1962). According to measurements made by Lelley (1966), when there is no wind wheat pollen travels 1 m and drops 55–60 cm in a second. By contrast, D'Souza (1970) found that the pollen grains travelled two metres in a second, while dropping only 19 cm. The spread of pollen in the air is greatly influenced by the wind. At a wind speed of 3 m/s (10.8 km/h) wheat pollen may travel 50 m and reach a height of 0.9 m. In investigations on the flowering biology of hybrid wheat, the extent of seed setting was examined on plots of male sterile wheat sown at various distances from the pollinating variety. Even on male sterile plants sown 20 m from the pollinating variety 0.6% seed setting was observed (De Vries, 1974). In experiments carried out by Nickerson Ltd. in England seed setting was observed as far as 150 metres downwind from the pollinating variety (Pickett, 1987). Virmani and Edwards (1983) reported finding viable pollen 1000 metres from the pollinating variety. These examples show that a considerable increase in the isolation distance would be required to ensure GM-free plants, and this could hardly be achieved in practice.

During the course of the vegetation period weeds must be controlled and satisfactory plant protection must be carried out, since both weed infestation (especially with quarantine weeds or those whose seeds are difficult to remove from wheat seed lots) and the mass appearance of plant diseases or pests make it impossible to produce high quality seed.

Plants which are not variety identical must be rooted out of the population prior to flowering and removed from the field. The seed production standards specify different numbers of variety-alien plants which can be permitted per sample area for various plant species and propagation grades. In the case of winter wheat in Hungary (MSZ 6353, 1998), for example, this number is at most 1 per 100 m² for Super Elite, 2 for Elite, 4 for First Grade and 10 for Second Grade.

In order to retain germination ability, harvesting must be carried out as soon as possible after maturity. The combine harvesters must be cleaned very carefully, since seeds which remain in the harvester are one of the most frequent causes of mechanical mixing. If the seeds need to be dried, it should be done at low (40°C) temperature.

The homogeneity of the variety and compliance with the seed multiplication regulations are officially inspected and recorded in the field. Only grain originating from fields which have passed this official inspection can be used as seed.

The crude seed yield is cleaned, fractioned, dressed and packed in special processing plants, carefully avoiding mechanical mixing. GM varieties must be transported, stored and processed separately, and the equipment used must be thoroughly cleaned both before and after use. Samples, the frequency and size of which are laid down in the standard, are taken from the seed to determine seed quality. Seed quality consists partly of the purity of the samples (ratio of broken seeds, organic and inorganic waste, alien seeds) and partly of the germination ability, or germination vigour, of the seeds. The seed must also have the stipulated moisture content. Seed found to be satisfactory both in the field and in the laboratory can be certified, and this is witnessed by a label attached to the sacks or a designation on the packaging. In the case of genetically modified varieties any label and/or document, official or otherwise, which is affixed to or accompanies the seed lot must clearly indicate that the variety has been genetically modified.

In the case of plant species where genetically modified varieties are also available on the market, the complete freedom of conventional varieties from GMs cannot be guaranteed even theoretically. All that can be expected is that the extent of mixing with GM seeds should not exceed an appropriate tolerance threshold. In the European Union this threshold is

- 0.3% in the case of cross-pollinated crops
- 0.5% in the case of self-pollinated crops and vegetatively propagated crops.

Considering the fact that contamination may occur during seed multiplication each year due to cross pollination and volunteer plants, while mechanical mixing may take place during harvesting, transportation and storage, it is extremely difficult to keep to these limits and is only possible if the initial multiplication stock has an extremely low level of contamination.

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DEVELOPMENT OF FEMALE AND MALE GAMETOPHYTES IN CEREAL SPECIES

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The first steps in studies on the female and male gametophytes of wheat involved the light microscope analysis of semi-thin sections of embryo sacs containing egg-cells developing *in planta*. The information thus obtained on the development of the egg-cell from its initial formation to maturity contributed to the successful isolation of egg-cells. The morphological and ultrastructural details of egg-cells isolated 3, 6, 9, 12, 15 and 18 days after emasculation were examined to determine the function of the female gametophyte and its suitability for micromanipulation. A sufficient number of gametoplasts in the right stage of development are required for the successful microinjection and *in vitro* fusion of egg-cells and male gametes. No data are yet available on the fusigenicity of wheat egg-cells in various stages of development. Various *in vitro* fertilisation and microinjection techniques could be of service in gamete fusion experiments aimed at the creation of interspecific and intergeneric hybrids which do not occur in nature due to sporophytic incompatibility. The results acquired in investigations on immature embryos can be used for the study of embryos developing from egg-cells fertilised *in vitro*.

Key words: egg-cell development, morphology of isolated egg-cells, pollen development, early embryo development, morphology of young embryos

Introduction

The study of the female and male gametophytes of agriculturally important cereal species raises many important questions of reproduction biology, including the possibility of developing viable hybrid progeny by the artificial fusion of isolated egg-cells and male gametes, and of growing plants from the embryos in cell or tissue culture. With the present experiments and observations it was hoped to contribute to the solving of these problems.

As a first step in studying the differentiation of the female and male gametophytes in cereal species, the development dynamics of the female gametophyte was investigated. The analysis of the morphology of the egg-cell is complicated by the location of the embryo sac within the ovary which makes it difficult to reach the egg-cell. Since the investigations of Timár et al. (1997) suggested that there was a certain degree of synchronisation between the development dynamics of the male and female gametophytes in wheat, the stage of development of the male gametophyte was used as an indicator, since these stages can be distinguished by a simple rapid method (carmine acetic acid staining). The developmental stages of the two types of gametophytes were

compared in samples taken at various times. In *Triticum* species with various ploidy levels changes over time in the size of the embryo sac could be clearly seen, and differences caused by the differentiation dynamics could also be observed (Timár et al., 1997). The first appearance of the egg-cell during cell formation in the embryo sac can be detected when the male gametophyte is in the Bn (binucleate) or Bc (bicellular) stage. The development and maturation of the egg-cell were recorded until anthesis.

These observations suggested the need for further investigations to clarify when the female gametophyte of wheat could be regarded as functionally mature. This information will be important in determining which phase of gamete development is best suited for various biotechnological manipulations.

In the second step changes in the size of the egg-cell were recorded in order to decide whether there was any correlation between the sizes of the egg-cell and embryo sac. The examinations covered the period from the first appearance of the egg-cell to the mature phase when the egg-cell reached the receptive stage.

Attention was then turned to changes occurring during the differentiation of the male gametophyte. The data proved that in addition to morphological differences, significant deviations in the size of the pollen grains could also be observed in various stages of development.

The investigations included the morphological, structural, cytological and physiological analysis of the maturation process of the egg-cell and the examination of two-week-old embryos from various wheat species, and from barley and rye. The size differences and morphological traits of the immature embryos provided useful information which could be used as guidelines in studies on intergeneric and interspecific hybrid embryos produced *in vivo* or as the result of biotechnological manipulations.

Materials and methods

Egg-cells developing in planta

Three wheat (*Triticum*) species with different genome compositions and ploidy levels, grown under small-plot field conditions, were used for the analysis of male and female gametophytes developing *in planta*. These species were as follows:

Triticum aestivum L. cv. Mv 15 (AABBDD) ($2n=6x=42$), *Triticum araraticum* Jakubz. (AAGG) ($2n=4x=28$) and *Triticum monococcum* L. (AA) ($2n=2x=14$).

For each species 10 main spikes in the same stage of development were isolated using cellophane bags and used to trace the *in vivo* development of the female gametophyte. Ten pistils were taken from each spike every day from the mid-uninucleate stage (Mu) until the development of three-celled pollen at anthesis. For the purpose of microtechnical analysis the pistils were fixed and stored in 2.5 % glutaraldehyde solution prepared using 0.1 M phosphate buffer. Egg-cell differentiation was studied under a Zeiss Ultraphot-III light microscope on semi-thin (1.5 μm) longitudinal sections prepared from pistils embedded in Spurr (1996) resin using traditional ultramicrotechnical techniques and stained with 0.5% toluidine blue (O'Brien and McCully, 1981). A series of sections prepared from pistils in different stages of development was examined to identify those in which egg-cells could be found at the micropylar end of the embryo sac. The radial size of the egg-cells (in μm) was then determined using an ocular micrometer.

The male gametophyte

After taking samples on the same days as described for egg-cells developing *in planta*, the anthers were fixed in Carnoy solution (3:1 mixture of alcohol and acetic acid). In the course of the examinations pollen grains from anthers in various stages of development were stained with carmine acetic acid and slides were prepared. These were examined under an OPTON 67275 microscope to determine the stage of microspore development using the nomenclature of Ouyang et al. (1973). The stage of development of 100 pollen grains was determined and their diameters were recorded. The data were statistically evaluated using analysis of variance.

Detailed data were obtained on the mature three-celled pollen of the spring wheat variety Chinese Spring. Ten pollen grains were chosen under the microscope from each of five anthers. Only those in which the generative and vegetative cells were clearly separated from each other (25 in all) were suitable for carrying out measurements. The data provided detailed information on the proportions of the cells of the male gametophyte, compared to each other and compared to the egg-cell.

Studies on the maturation of the egg-cell

The fine structure, size changes and morphological features of the wheat egg-cell were studied on the hexaploid spring wheat variety Chinese Spring. The plants were grown in a phytotron growth chamber (Conviron, PGV type) using the t2 spring climate programme described by Tischner et al. (1997). The egg-cells were excised under sterile, controlled conditions. Since the development of the egg-cell was examined in comparison with the development dynamics of the male gametophyte, it was necessary to emasculate the androgynous flowers at the required stage of pollen development (Bn = binucleate stage). The determination of this developmental stage was carried out using an OPTON 67275 microscope after carmine acetic acid (1%) staining.

The wheat egg-cells were mechanically isolated from the ovaries of emasculated flowers 3, 6, 9, 12, 15, 18 and 21 days after emasculation (Kovács et al., 1994) under a ZEISS STEMI 2000C binocular microscope. The ovaries were removed from the spikes and cut in half under a stereomicroscope so that the micropylar end of the ovules, containing the embryo sac, could be separated from the surrounding ovary tissues. The egg-cells were then released using a microneedle and were floated into isosmotic (0.6 M mannitol) isolation solution containing 2 mmol $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. The isolated egg-cells (Pónya et al., 1999) were then placed in 5 μl drops of mannitol solution using a WPI A203XVZ nanolitre injector. In order to avoid evaporation, the microdrops were covered with paraffin oil. The gametic cells were then collected from the microdrops using a nanolitre injector and embedded in agarose drops prepared by mixing equal proportions of 1.2 M mannitol solution and 2% low melting point agarose gel (SIGMA, op = 30°C, fp = 65°C, No. A-9414). The slides containing the agarose drops were placed in a refrigerator at 4°C to accelerate solidification. The egg-cells were then fixed for 12 hours in 0.2 M Na cacodylate $[(\text{CH}_3)_2\text{As}(\text{O})\text{ONa} \cdot 4\text{H}_2\text{O}]$ buffer (pH 6.9) containing 2.5% glutaraldehyde. The diameter of morphologically intact cells and cell nuclei which had kept their shape was measured using an ocular micrometer. A total of 60 female gametes were obtained from the genotype examined. The samples taken on the 21st day could not be evaluated due to the severe degradation of the egg-cells. The egg-cells were embedded in artificial resin for light microscope and TEM studies.

Two-week-old embryos

The embryo development in wheat species with different ploidy levels and genome compositions was compared using the same plants from which egg-cells developing *in planta* were extracted. The septicellular (octonucleate) embryo sac was compared with the two-week-old embryo and the mature embryo.

Morphological analyses on immature embryos were also carried out on the barley variety Igri and the rye variety Lovászpatonai 407.

Three spikes on each of the plants, grown as described above, were emasculated and artificially pollinated. On the 14th day after pollination the two-week-old ovules were fixed in 2.5% glutaraldehyde solution. The embryos were removed under a stereomicroscope (ZEISS Stemi 2000-C) and placed on 1:1 LMP (SIGMA, op = 30°C, fp = 65°C, No. A-9414) agarose-glucose drops on slides, then sandwiched when semi-solidified with another drop. When the

agarose had completely solidified a razor-blade was used to cut the block containing the embryo out of the drop. These blocks were stored in 2.5% glutaraldehyde solution until further analysis.

The length and width of embryos embedded and stored using this agarose sandwich block method, which protected them from damage and drying out, were determined under a microscope with an ocular micrometer. For morphological comparison the two-week-old embryos were stuck on a grid after fixation and dehydration and coated with gold. They were then examined under a scanning electric microscope (Zeiss EM910) at 60 kV. Digital photographs were prepared using an image processing program (SOFT IMAGING SYSTEM 3.0).

In order to obtain mature embryos, mature grains of the three *Triticum* species were soaked in distilled water at room temperature for 48 hours. After splitting the seed-coat the embryos were carefully removed from the swollen grains under a stereomicroscope. The embryos were then measured and the results were compared with those obtained for the embryo sac and the 14-day-old embryos (Table 5).

Results

Changes in egg-cell size

The observations made on the maturation processes of egg-cells developing *in planta* showed considerable differences in size both over the 7-day interval and between the species (Table 1). When the male gametophyte was in the Bn or Bc stage of development, the egg-cells measured 14–17 μm , but when the male gametophyte matured, at anthesis, the size of the egg-cell ranged from 40–58 μm , indicating a 3–4-fold increase in size from formation to the receptive stage. The mature egg-cells of hexaploid wheat were the largest (58 μm), while those of tetraploid and diploid wheat measured 42.5 μm and 40.5 μm . In experiments carried out on wheat, Maksimov (1972) established the fact that as the ploidy level increased there was also an increase in the size of the pollen grains and ovary. On the 7th day of the study the egg-cells of species with lower ploidy levels were far smaller than those of the hexaploid, but did not differ substantially from each other. In the course of maturing the egg-cell also changes shape (Fig. 1). The initial state can be seen in Figure 1a and b. The pear-shaped form can be clearly observed in Figure 1c, d and e, while the mature, receptive egg-cell is visible in Figure 1e and f. Apart from the differences in size, no important morphological differences were observed between egg-cells of species with different ploidy levels when semi-thin sections made in the same stage of development were examined. These differences in the size of the egg-cells were in agreement with those observed during the development of the embryo sac.

Maturation of the egg-cell

The cytology, ultrastructure and size changes characteristic of the maturation of egg-cells in the hexaploid wheat variety Chinese Spring were studied and evaluated as a function of age.

The horizontal and vertical diameters of the egg-cells were measured 3, 6, 9, 12, 15 and 18 days after emasculation. Although egg-cells were also excised 21 days after emasculation, these were so degraded that the cell membranes were damaged. Egg-cells take up a round shape after isolation; *in planta* they are located between the synergids and are pear-shaped. It can be seen from the data that there is no great difference between the horizontal and vertical diameter, so the egg-cells can be regarded as spherical.

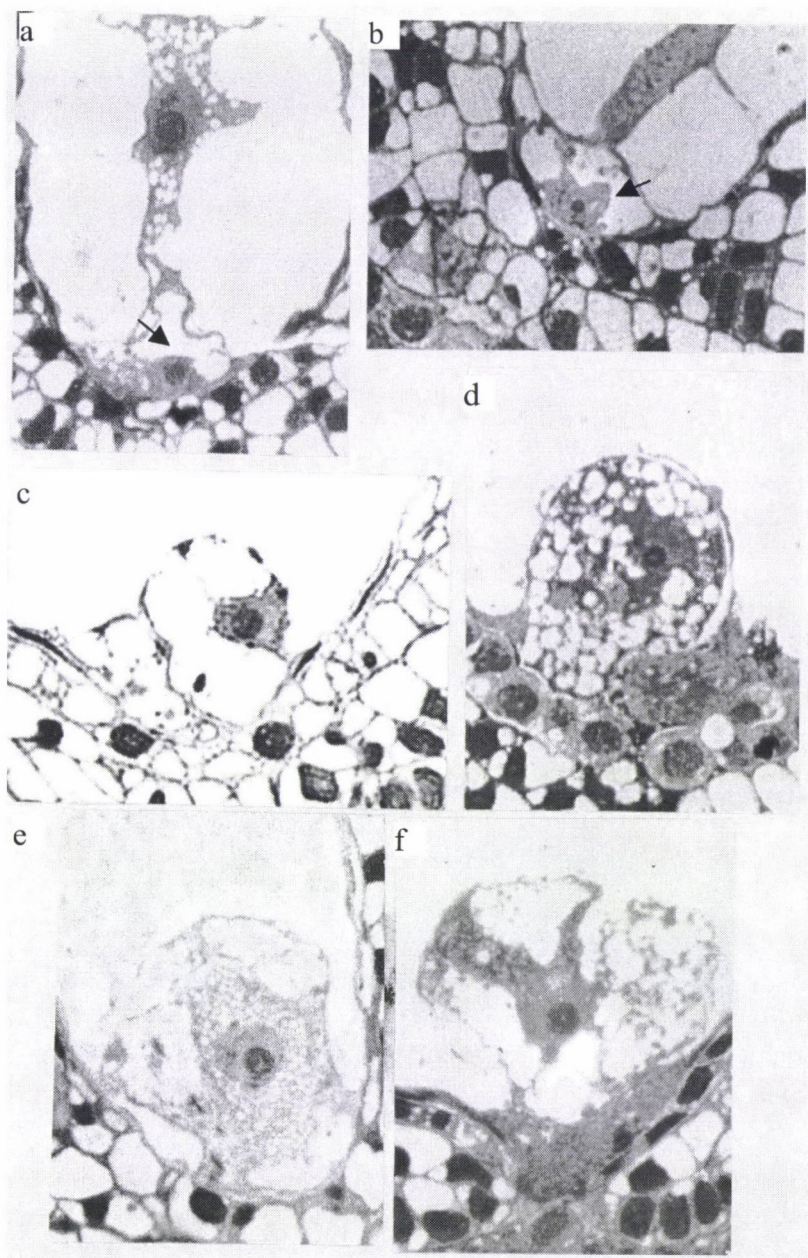


Fig. 1. Development of the egg-cell of *T. aestivum* cv. Mv15 from the seven-celled stage to maturity at anthesis; a: Egg-cell at the beginning of the seven-celled stage; b: With an increase in the quantity of cytoplasm, the egg-cell starts to grow; c: The egg-cell gradually becomes pear-shaped; d: Pear-shaped egg-cell in the embryo sac, and the differentiation of the cell organelles; e: The pear shape and the darkly stained cell nucleus are clearly visible; f: Mature, receptive egg-cell; arrows indicate the egg-cell

Table 1
Diameter of egg-cells (μm) in wheat species with different ploidy levels

Days	<i>T. aestivum</i> cv. Mv 15 (hexaploid)	<i>T. araraticum</i> (tetraploid)	<i>T. monococcum</i> (diploid)
2	14.5	—	—
3	46.4	14.5	—
4	49.3	20.3	17.4
5	50.7	34.8	34.8
6	43.5	29.0	29.0
7	58.0	42.5	41.05

Due to the nature of section preparation, and the very low number of sample replications, LSD values could not be calculated.

In the case of Chinese Spring the mean diameter of 3-day-old egg-cells was 60.91 μm . This increased consistently until the 18th day, when it measured 85.86 μm (Table 2). The diameter on the 6th to 9th days, which are critical for fertilisation, ranged from 63.49 μm to 73.91 μm . The cell nuclei attained their maximum diameter (14.17 μm) on the 15th day after emasculation, after which the diameter decreased. The size of the cell increased at a greater rate than that of the cell nucleus. According to observations reported by You and Jensen (1985) the size of the wheat egg-cell varied between 50 and 70 μm .

Structural analysis of egg-cells of Chinese Spring

Egg-cells three days after emasculation

The cell was small, and small vacuoles could be seen evenly distributed in the cytoplasm. The organelles (mitochondria, plastids) were arranged in a semi-circle round the cell nucleus, which was clearly visible and contained a single nucleole. The cell membrane was susceptible to damage during isolation (Fig. 2a).

Table 2
Mean diameters (μm) of egg-cells and egg-cell nuclei (μm) in the wheat variety Chinese Spring

Days	Cell diameter	Nucleus diameter	Difference
3	60.92	9.77	51.15
6	66.01	10.55	55.46
9	73.92	10.69	63.23
12	74.40	11.82	62.59
15	83.35	14.18	69.17
18	85.87	13.52	72.35
LSD _{5%}	3.65	1.02	—
LSD _{1%}	4.82	1.66	—

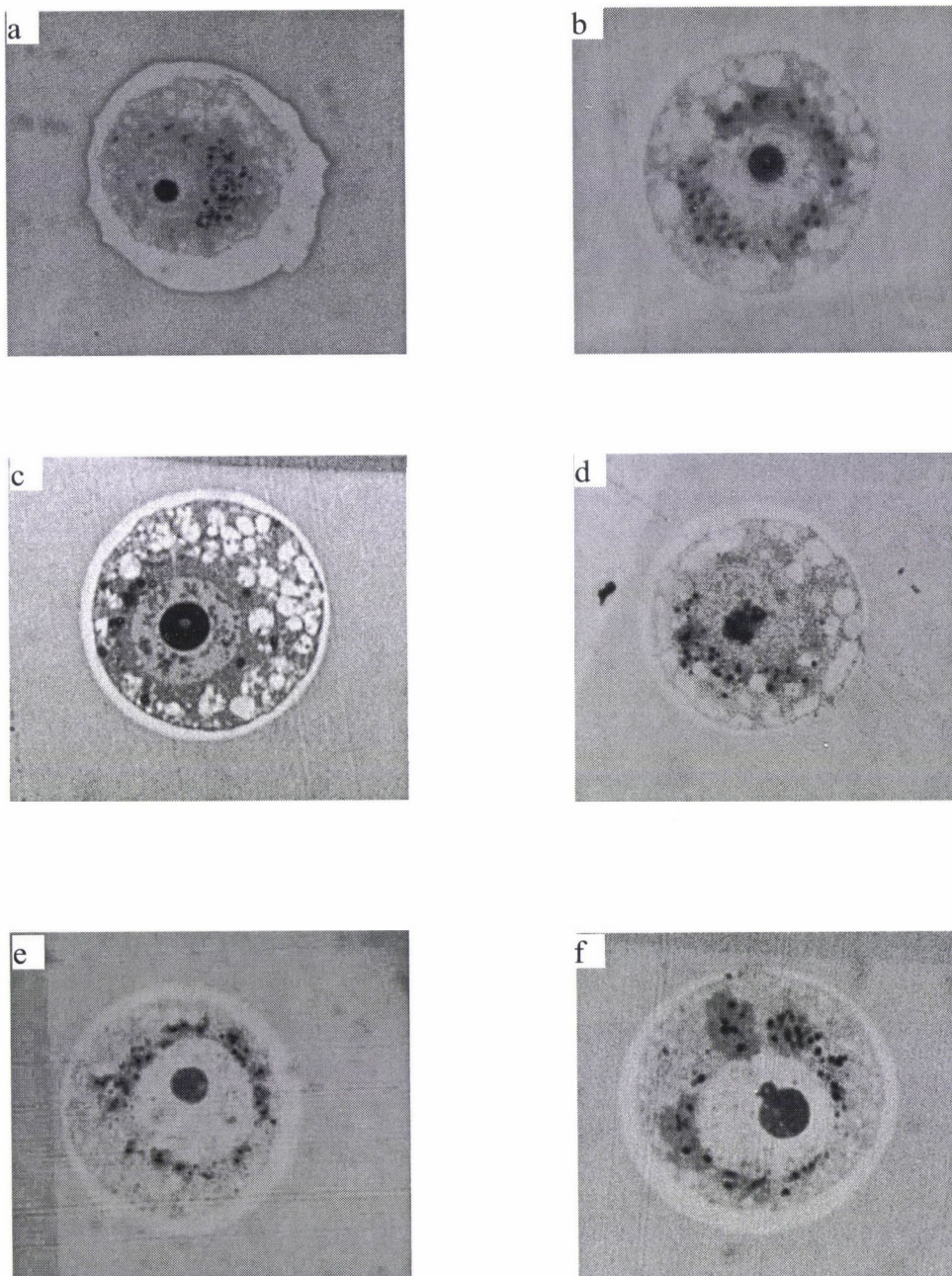


Fig. 2. Structure of isolated egg-cells of the wheat variety Chinese Spring; a: 3-day-old egg-cell; b: 6-day-old egg-cell; c: 9-day-old egg-cell; d: 12-day-old egg-cell; e: 15-day-old egg-cell; f: 18-day-old egg-cell.

Egg-cells six days after emasculation

Both the cell and the cell nucleus had grown and the nucleole was more distinct. The organelles formed a full circle round the nucleus. The vacuoles in the cytoplasm had begun to merge and were thus considerably larger. The cell nucleus was heterochromatic and ready for fertilisation. Nucleolar vesicles could be seen in the nucleole, indicative of rRNA synthesis. This in turn demonstrated that the nucleus was active (Fig. 2b).

Egg-cells nine days after emasculation

The cytoplasm had become denser. The organelles were close to the nucleus, but were located at one pole of the cell. Small vacuoles were again to be seen in the cytoplasm. The nucleole had grown and contained nucleolar vesicles. The greatest difference from the six-day-old egg-cells was the polarised location of the organelles, which could also be found in the peripheral part of the cytoplasm. Their radial distribution had become random, but only at one of the poles (Fig. 2c).

Egg-cells twelve days after emasculation

The volume of the nucleus had grown and the vacuoles were becoming fused. The organelles no longer surrounded the nucleus, but were all on one side. The nucleole was granulated and contained a large number of vesicles (Fig. 2d).

Egg-cells fifteen days after emasculation

The vesicles of the nucleole had begun to fragment, indicating that rRNA was beginning to leave the nucleole. The organelles were located in a circle round the nucleus. The vesicles were smaller and the volume of the vacuoles had decreased. The nuclear membrane was less distinct (Fig. 2e).

Egg-cells eighteen days after emasculation

The nucleole was large and the vesicles appeared to be loosely attached to the nucleolar membrane. The nucleus was surrounded by pockets of cytoplasm which contained the organelles. The cell nucleus had decreased in size, and the diameter of the cell to a lesser extent (Fig. 2f).

These observations are in agreement with the results of flowering biology research carried out by Rajki (1961), who reported that the stigma exhibited very little receptiveness in the very early stages of development (during the differentiation of the stigma lobes); very little seed setting was achieved when such flowers were pollinated. Four to six days after emasculation the development of the stigma was sufficient for the achievement of a high seed setting percentage, while 12–14 days after emasculation no seeds were set due to the senescence of the stigma. Rajki did not examine the developmental stages of the female gametophyte (embryo sac and egg-cell). Molnár-Láng and Rajki (1983) carried out histological analyses on seeds developing after fertilisation at the beginning and end of flowering.

After isolation the wheat egg-cell, which is pear-shaped under *in situ* conditions (You and Jensen, 1985) assumes a spherical shape due to changes in the osmotic pressure, and probably to the lack of the natural tissue environment and the pressure exerted by neighbouring cells *in planta*.

Isolated egg-cells are usually spherical protoplasts both in the young and old stage. On semi-thin sections (0.5–1.0 μm) young egg-cells usually exhibit peripherally located vacuoles and a large nucleus with a single nucleole, all surrounded by dense cytoplasm. The volume of the egg-cell gradually increases as it matures. The mean volume of egg-cells in early developmental stages was found to be 96,917 μm^3 , which increased to 135,900 μm^3 12 days after anthesis. It can be seen from the data that the wheat egg-cell is larger than isolated maize egg-cells, which were found to have a volume of 62,418 μm^3 at anthesis (Faure et al., 1992).

The light microscope examinations were confirmed by TEM analysis (Pónya et al., 1999). Lipid bodies, mitochondria, amyloplasts and starch granules could be seen among the peripheral vacuoles in egg-cells isolated 3 days prior to anthesis. The structure of the aging egg-cell exhibits a number of characteristic features. Degradation can be observed in the chromatin in the cell nucleus. Chromatin fragments become attached to the cell nucleus membrane, leading to the development of membrane blebbing. A large quantity of lipid bodies, starch granules and protein is accumulated in the cytoplasm. The mitochondria have normal structure, but lysis can be detected in the vesicles and autophagous vacuoles have formed. These can be explained by the syndrome of programmed cell death, which can be observed in aging egg-cells and has also been reported in animal cells (Cohen, 1993) and in plant suspensor cells (Jones and Dangl, 1996; Gray and Johal, 1998).

Structural analysis on receptive egg-cells (Fig. 3) confirmed earlier observations that the female gametophyte gradually loses its receptiveness approximately 2 weeks after anthesis. Receptive nine-day-old egg-cells, which are the closest in time to anthesis (Fig. 3a–e), are characterised by intense polarisation and pronounced staining. Ultrastructural analysis shows a dense cytoplasm, a uniform nuclear membrane (Fig. 3b) and a large number of mitochondria (Fig. 3d). Evenly distributed cell organelles and lipid drops can be observed in the cytoplasm (Fig. 3c). The endoplasmic reticulum engulfing the nuclear envelope was also observed by Naumova and Matzk (1998). The endoplasmic reticulum plays an important role in the life of the cell during the maturation process, so changes in its structure could be indicative of a change in function.

Male gametophyte

The development of the male gametophyte was examined in three developmental stages (Mu: mid-uninuclear microspore, Bn: binuclear microspore, Tc: three-celled mature pollen) based on the diameter of the pollen grains. The data were analysed by analysis of variance (Table 3). Great deviations were found in the size of the pollen grains in the three developmental stages. The deviations between *Triticum* species with different ploidy levels were significant at the $p = 1\%$ level. A correlation between pollen size and ploidy level was reported in wheat by Maksimov (1972) and in barley by Johansen and von Bothmer (1994), who found that the size of the pollen rose with the ploidy level. Pfahler et al. (1982) examined the effect of genotype, ploidy level and genetic background on rye pollen and found a clear correlation between the ploidy level (1n, 2n) and the pollen size (volume). Correlation analysis proved that the ploidy level had a greater effect than the genotype.

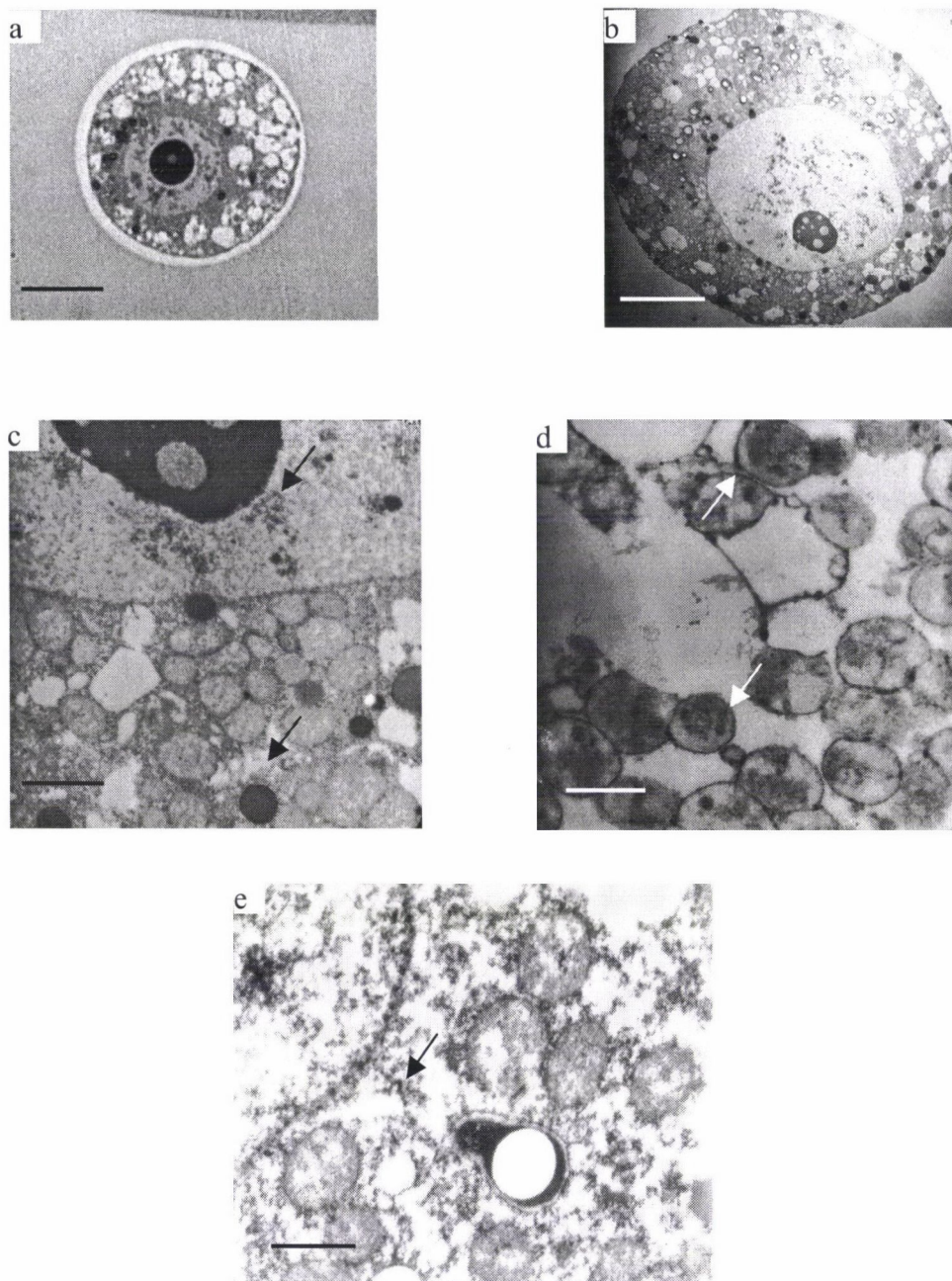


Fig. 3. Isolated receptive wheat protoplast; a: Semi-thin section of a receptive egg-cell three days after anthesis. Bar: 17.4 μm ; b: TEM image of the receptive protoplast, with a uniform nuclear membrane and uniformly distributed vacuoles. Bar: 9.6 μm ; c: Detail of the cell nucleus with chromatids and lipid drops (arrows). Bar: 12.5 μm ; d: Detail of the mitochondria (arrows). Bar: 1.08 μm ; e: The endoplasmic reticulum is attached to the nuclear membrane (arrow). Bar: 0.97 μm .

Table 3
Analysis of the variance of pollen sizes (μm)

Genotype	<i>In vivo</i>			<i>In vitro</i>		
	Mu	Bn	Tc	Mu	Bn	Tc
<i>T. aestivum</i> Mv 15 (hexaploid)	41.05	47.12	55.48	41.05	46.64	47.70
<i>T. araraticum</i> (tetraploid)	31.68	38.85	44.75	31.58	35.02	43.65
<i>T. monococcum</i> (diploid)	29.35	40.92	45.80	29.35	34.36	35.64

LSD_{5%} = 1.2 ; LSD_{1%} = 1.5; between any two combinations; Mu: mid-uninucleate microspore; Bn: binuclear microspore; Tc: three-celled mature pollen

A comparison of the effect of *in vivo* and *in vitro* environments on the size of the pollen grains showed significant differences for all the species at all stages of development, except in the case of *T. aestivum* in the Bn stage and of *T. araraticum* in the Tc stage. When the three species were compared in the *in vivo* environment there was no significant difference between *T. araraticum* and *T. monococcum* in the Tc stage of development (mature pollen). Within the *in vitro* environment the only differences that were not significant were those between the Bn and Tc stages in *T. aestivum* and between *T. araraticum* and *T. monococcum* in the Bn stage. The difference between the Bn and Tc stages in *T. monococcum* was significant at the $p = 5\%$ level. For all the species, pollen matured *in vitro* was smaller at all stages of development than the corresponding pollen matured *in vivo*.

T. aestivum had the largest mature pollen in both environments (55.48 μm ; 47.70 μm), while that of *T. monococcum* was the smallest in the Tc (35.64 μm) and Mu (29.35 μm) stages.

Studies on the mature pollen of the hexaploid wheat Chinese Spring

The data obtained for mature, three-celled pollen of Chinese Spring are summarised in Table 4. The diameter of mature pollen was 55.7 μm , which was similar to that of the hexaploid wheat variety Mv 15 (58 μm). The male gametes in the pollen grain were only about a quarter of the length of the mature egg-cell.

The average size of the two gametes in the mature pollen was 16 μm and 15.2 μm . There was a consistent difference in the size of the two gametes, though this difference was not statistically significant. The diameter of the vegetative cell was 13.9 μm and that of the cell nucleus 6.9 μm . It also differed from the gametes in shape. The size of the vegetative cell was significantly different from that of both gamete cells.

Young embryos of cereal species

Two-week-old excised embryos were compared with the volume of the embryo sac at pollen shedding and with those of embryos or germs removed from mature pollen grains soaked in distilled water (Table 5).

Table 4
Size of male gametophyte components in wheat variety Chinese Spring (μm)

Male gametophyte components	Size
Pollen diameter	55.7
Generative cell (1)	16.0
Generative cell (2)	15.2
Vegetative cell	13.9
Vegetative cell nucleus	6.9

LSD_{5%} = 1.33; LSD_{1%} = 1.76; between any two combinations

Table 5
Volume of the embryo sac (μm^3) and embryos (μm^3) of wheat species with various ploidy levels

Ploidy level	Embryo sac	Two-week-old embryo	Germ or embryo (mature seed)
Hexaploid	1.27E+7	68.70E+7	611.0E+7
Tetraploid	0.02E+7	24.60E+7	416.0E+7
Diploid	0.03E+7	87.70E+7	597.0E+7
LSD _{5%}	0.23E+7	7.8E+7	112.0E+7
LSD _{1%}	0.52E+7	10.8E+7	154.0E+7

The hexaploid embryo sac was found to be considerably larger at anthesis than those of tetraploid and diploid species. There was no significant difference between the latter. When the size of the embryo sac was compared with that of the two-week-old embryo, considerable differences were observed. The embryo sac was largest in hexaploid wheat, so the embryo exhibited a 54.5 times increase in volume compared with this. This increase was far greater in the tetraploid (1230 times) and greater again in the diploid (2923 times).

The data reflect a remarkably intense development for the embryo of diploid wheat. The size of the two-week-old diploid embryo was significantly greater than that of the hexaploid embryo. This difference was smaller and non-significant in the mature embryo stage. The two-week-old embryo of tetraploid wheat was the smallest of all, and this significant difference remained in the mature stage.

Mature seeds were also compared with the two-week-old embryos and it was found that for hexaploid wheat the size of the two-week-old embryo was 48% that of the mature seed, while this figure was only 22.8% for the tetraploid, but 58% for the diploid. For rye the two-week-old embryo was 29.3% as large as the mature seed, while this ratio was smallest (19.5 %) for barley. These data could be extremely useful in studies on embryos developing after *in vitro* fertilisation.

The shape of the embryo exhibited great variability (Fig. 4a–e). The hexaploid embryo was rectangular, though the scutellum was rounded at the corners (Fig. 4a). The tetraploid was triangularly pointed towards the suspensor cell (Fig. 4b), while the diploid was round at the suspensor end and narrowed

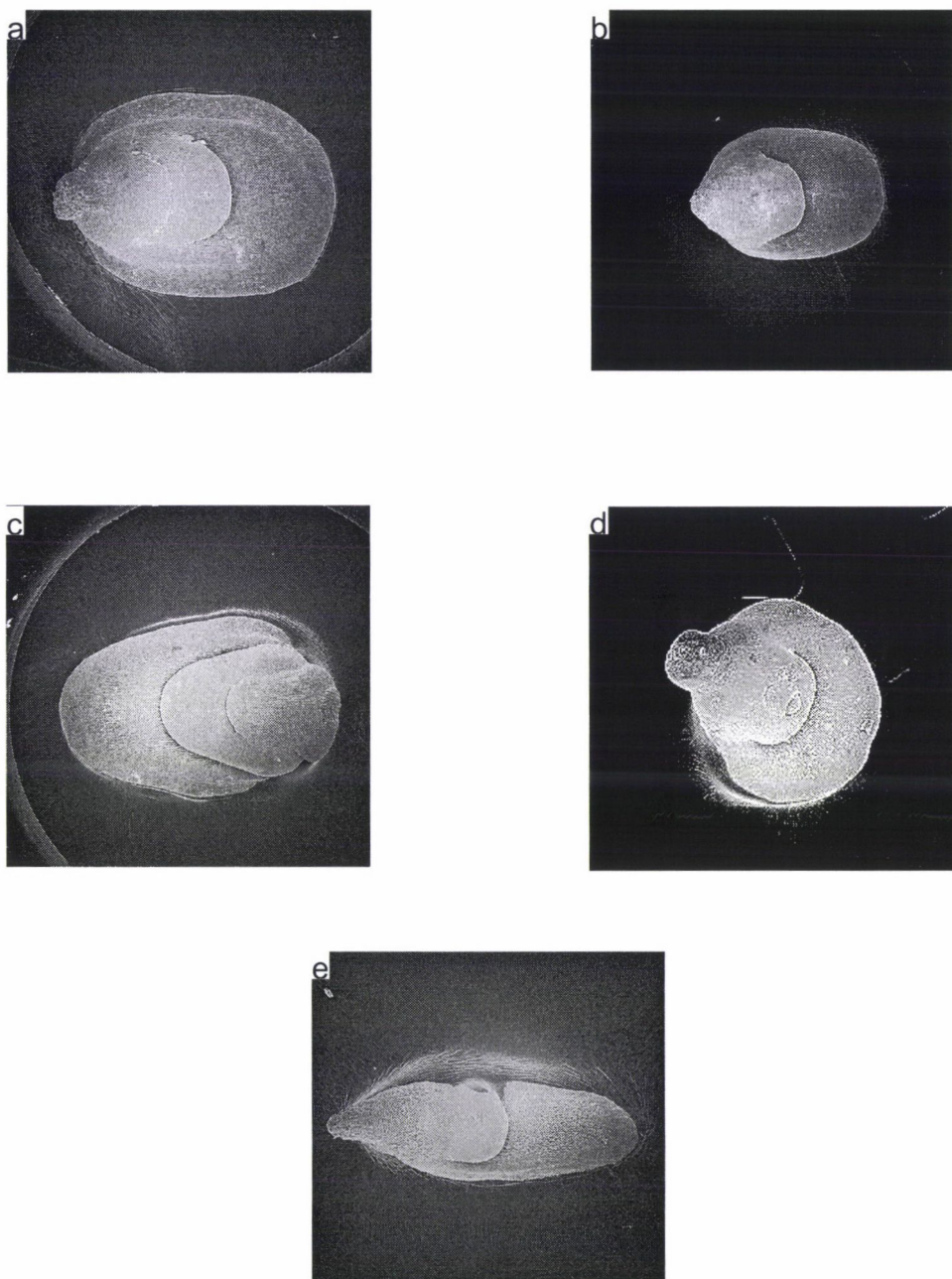


Fig. 4. Two-week-old embryos of various cereal species. a. *Triticum aestivum* cv. Mv15, the embryo is rectangular, the scutellum is rounded at the corners of the rectangle; b. *T. araraticum*, the embryo is pointed at the suspensor end; c. *T. monococcum*, the embryo is round at the suspensor end and narrows towards the scutellum; d. *Hordeum vulgare* cv. Igri, the scutellum is round; e. *Secale cereale* cv. Lovászpatonai, the embryo forms an elongated ellipse

towards the scutellum (Fig. 4c). In barley the scutellum was round (Fig. 4d), while the embryo of rye had an elongated elliptical shape (Fig. 4e). The shape of the embryo is thus characteristic and could be just as useful for distinguishing between the species as external traits such as grain colour.

Detailed information on the structural and physiological or biochemical changes taking place in the various cells of the embryo sac during the period from macrosporogenesis to flowering are only available for a very limited number of *Gramineae* species (Bennett et al., 1973; Russell, 1979; Cass and Peteya, 1986; Dow and Mascarenhas, 1991). Previous studies have concentrated primarily on the formation of the cell walls (You and Jensen, 1985).

No precise knowledge has yet been obtained on whether the embryo sac can be fertilised (artificially) at the beginning of the seven-celled stage or on which developmental phase of the gametophytic cells is the most suitable for manipulation experiments. Recent publications have reported the successful fusion of gametoplasts in various species (Kranz et al., 1995; 1996). The results achieved so far justify the continuation of the present cell biology research in order to investigate the developmental stages of male and female gametophytes in species intended for use in gametosomatic crosses.

The present results will promote the successful continuation of the *in vitro* wheat fertilisation programme (Kovács et al., 1994; 1995). The morphological observations made on the embryo sac will facilitate the mechanical isolation of living egg-cells and the isolation of gametes in the best stage of physiological development for fusion. This could lead to an improvement in the frequency of successful gamete fusion and in the plant regeneration ability of fusion products.

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EFFECTS OF HERBICIDES ON NODULATION AND GROWTH OF TWO VARIETIES OF PEAS (*PISUM SATIVUM*)

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The effects of the pre-emergence application of terbutryn/terbuthylazine at 1.40 and 2.80 kg a.i./ha and prometryn at 1.70 and 3.40 kg a.i./ha and the post-emergence application of bentazone at 1.44 and 2.88 kg a.i./ha were studied on nodulation, root and shoot growth in two pea varieties, namely Rex and Guido. Of these the lower rates are the recommended rates for field application. Bentazone even at the recommended rate decreased nodule dry weight, whereas all three herbicides at double the recommended rate (except terbutryn/terbuthylazine in Rex) decreased the number and dry weight of nodules. Terbutryn/terbuthylazine and bentazone decreased root dry weight to a greater extent, whereas prometryn had only a slight effect. Shoot growth was adversely affected by all three herbicides in both the varieties, but prometryn and bentazone had less adverse effects in Rex than in Guido. Compared to the recommended rate, the herbicides had a greater adverse effect on nodulation at double the recommended rate.

Key words: growth, herbicides, nodulation, peas, varieties

Introduction

Like any other crop, varieties of peas may differ in their sensitivity to the herbicides used for controlling weeds under field conditions. Based on this criterion a herbicide can only be sprayed to control weeds in varieties which show tolerance to the herbicide (Gane et al., 1984). The sensitive varieties may show toxicity symptoms. Sometimes the application of a herbicide even to a recommended variety may show adverse effects on the growth of the crop. This can happen due to changed weather conditions after the application of the herbicide. In cases where a herbicide results in phytotoxicity it may affect not only aboveground plant growth but underground growth as well, including nodulation. Furthermore, nodulation is controlled by many plant, rhizobial and environmental factors. In peas the genotype of the host plays a greater role in determining effective symbiosis than the bacterial strain (Fesenko et al., 1994). Herbicides as well as varieties need to be screened in order to find suitable chemicals and varieties for the achievement of higher productivity coupled with better symbiotic parameters. The studies reported in this paper tested the effects of three herbicides at two rates of application (recommended and double the recommended field application rate) on the nodulation, growth and yield (pod weight) of two spring pea varieties. Among the herbicides tested, two (terbutryn/terbuthylazine and prometryn) belong to the chemical family of triazines and are applied pre-emergence, whereas the other herbicide, bentazone, belongs to the chemical family of benzothiadiazoles and is applied post-emergence.

Materials and methods

Site description

The experiment was conducted in a glasshouse without any supplementary lighting or heating at Henfaes Farm, University of Wales, Bangor, Gwynedd, United Kingdom. As the experiment was conducted in the glasshouse it is expected that temperatures were higher than outside.

Treatments and experimental design

The effects of six herbicide treatments along with an unsprayed control (Table 1) on two spring pea varieties, namely Rex and Guido, were tested in a pot experiment sown in a medium-textured (sandy clay loam) mineral soil of the Denbigh series in a factorial randomised complete block design having three replicates. Rex is a normal-leaved variety having a 1000-seed weight of 228 g (NIAB, 1996). It is a white pea type with an average straw length of 86 cm. Guido, also a normal-leaved variety, has a 1000-seed weight of 340 g. It is a marrowfat type variety with a straw length of 76 cm.

The herbicide Opogard is a mixture of 350 g terbutryn + 150 g terbutylazine/litre, whereas Gesagard contains 500 g prometryn/kg and Basagran contains 480 g bentazone/litre. The herbicides Opogard and Gesagard are manufactured by Ciba-Geigy, whereas Basagran is manufactured by BASF. The herbicides were applied using a laboratory hand sprayer. Terbutryn/terbutylazine and prometryn were applied one day after sowing, whereas bentazone was applied 12 weeks after sowing when the plants tested safe to the pea leaf wax test (Gane et al., 1984; PGRO, 1993).

Plant husbandry

The plants were grown in five-litre plastic pots, each having a surface area of 342 cm². Two fungicide (thiram)-treated seeds were sown in each pot on 12 February 1996. After emergence only one plant per pot was retained. The seeds were not inoculated prior to sowing as British soils have enough rhizobia for peas (Gane et al., 1984). Phosphorus and potassium at the rate of 72 kg each (as P₂O₅ and K₂O) per ha were applied before sowing in the form of a compound Champion Fertiliser (0-24-24). No nitrogenous fertiliser was applied. The plants were watered with tap water as and when required. Weeds were removed manually in all pots whenever necessary. The plants were provided support with sticks to keep them upright.

Observations recorded

The visual effects of pre-emergence herbicides on plants were recorded 4, 6 and 8 weeks after application. The effects of bentazone were recorded 2 and 3 weeks after its application. The plants were harvested 16 weeks after sowing when they were just past the seed-filling stage (growth stage code 207; Knott, 1987). Stem + leaves together and pods (along with seeds) separately were put in paper bags and their dry weight was recorded after drying the samples in an oven for 48 h at 80°C. The roots (along with attached nodules) were removed from the soil very carefully. The finer roots, where feasible, were also collected. The roots were washed in water in a sieve to remove the attached soil. The roots were dried with tissue paper and nodules were detached before drying the roots in paper bags in a drying oven. The nodules were of varying sizes. Depending upon their size they were divided, visually, into three categories, namely, small, medium-sized and large (with average diameter of less than 3 mm, between 3 and 7 mm, and more than 7 mm, respectively), and were counted. Nodules of all sizes were then combined together and were dried in a drying oven at 70°C for 48 h before recording their dry weight.

Statistical analysis

The data were analysed in a factorial randomised complete block design using the Minitab statistical package version 10.51. For treatments having a significant F value a least significance difference was calculated by Tukey's method.

Table 1
Treatments applied in varietal response experiment

Herbicides		Rate (kg a.i./ha)	Relevance to field application rate
Active ingredient	Product		
Terbutryn/terbuthylazine	Opogard	1.40	Recommended
		2.80	2 × recommended
Prometryn	Gesagard	1.70	Recommended
		3.40	2 × recommended
Bentazone	Basagran	1.44	Recommended
		2.88	2 × recommended
Unsprayed control			

Results

Visual symptoms on plants

The pre-emergence herbicides at recommended and double the recommended field application rate did not cause any visual phytotoxicity to either of the varieties. However, post-emergence application of bentazone at double the recommended rate caused yellowing of plants, with more effect on Guido than on Rex.

Number of nodules per plant

The majority of the nodules were small-sized in all the herbicide treatments and in both the varieties (Table 2). The proportion of medium- and large-sized nodules was very small. Guido had significantly more nodules in the medium- and large-sized categories than Rex. Overall Guido had 22% more total nodules than Rex. Bentazone at double the recommended rate decreased the total number of nodules significantly. Terbutryn/terbuthylazine did not affect the total number of nodules but prometryn decreased their number at the highest rate of application. At double the recommended rate of application terbutryn/terbuthylazine, prometryn and bentazone decreased the total number of nodules by 2, 35 and 55%, respectively, compared to the unsprayed control. Although the herbicide × variety interaction was not significant except for the number of large nodules, the results provided some evidence that the effects of herbicides at double the recommended rate were greater on Guido than on Rex. At double the recommended rate terbutryn/terbuthylazine, prometryn and bentazone decreased the total number of nodules by 0, 28 and 41% in Rex and 21, 39 and 64% in Guido.

Table 2
Effect of herbicides on number of nodules in two pea varieties

Herbicides	Rate (kg/ha)	Varieties		
		Rex	Guido	Mean
<i>No. of small nodules/plant</i>				
Terbutryn/terbuthylazine	1.40	19.3	22.3	20.8
	2.80	26.3	20.6	23.5
Prometryn	1.70	22.0	18.6	20.3
	3.40	14.0	19.6	16.8
Bentazone	1.44	21.6	30.0	25.8
	2.88	13.6	10.6	12.1
Unsprayed control		16.4	22.8	19.6
Mean		19.0	20.6	
<i>No. of medium-sized nodules/plant</i>				
Terbutryn/terbuthylazine	1.40	9.0	10.0	9.5
	2.80	4.0	4.3	4.1
Prometryn	1.70	2.3	4.0	3.1
	3.40	4.0	2.6	3.3
Bentazone	1.44	1.3	3.0	2.1
	2.88	1.0	2.6	1.8
Unsprayed control		5.2	9.6	7.4
Mean		3.8	5.1	
<i>No. of large nodules/plant</i>				
Terbutryn/terbuthylazine	1.40	0.3	5.0	2.6
	2.80	1.0	4.6	2.8
Prometryn	1.70	0.0	3.6	1.8
	3.40	0.0	0.3	0.1
Bentazone	1.44	3.0	0.6	1.8
	2.88	0.0	0.3	0.1
Unsprayed control		3.3	4.7	4.0
Mean		1.0	2.7	
<i>Total number of nodules/plant</i>				
Terbutryn/terbuthylazine	1.40	28.6	40.6	34.6
	2.80	31.3	29.6	30.5
Prometryn	1.70	24.3	26.3	25.3
	3.40	18.0	22.6	20.3
Bentazone	1.44	26.0	33.6	29.8
	2.88	14.6	13.6	14.1
Unsprayed control		24.9	37.3	31.1
Mean		23.9	29.1	

		Nodule size			
		Small	Medium	Large	Total
S.E. (D.F.=26)	Herbicides (H)	3.31	1.02	0.38	3.78
	Varieties (V)	1.77	0.54	0.20	2.02
	H × V interaction	4.69	1.45	0.54	5.34

Dry weight of nodules per plant

Nodule dry weight was significantly decreased at double the recommended rates of terbutryn/terbuthylazine and prometryn and at both rates of application of bentazone (Table 3). The decreases in nodule dry weight at double the recommended rates of terbutryn/terbuthylazine, prometryn and bentazone were 30, 40 and 56%, respectively, whereas the corresponding decreases in nodule dry weight at the recommended rates were 3, 10 and 32%. The data thus show that terbutryn/terbuthylazine had the least and bentazone the highest effect on nodule dry weight. Averaged over the herbicide treatments Guido had significantly higher (110%) nodule dry weight compared to Rex. The herbicide \times variety interaction was significant. In both varieties and all herbicide treatments nodule dry weight was lower at the double rate than at the recommended rate of application.

Dry weight of roots per plant

Terbutryn/terbuthylazine at both rates and bentazone at double the recommended rate decreased root dry weight significantly (Table 3). At the recommended rates terbutryn/terbuthylazine and bentazone decreased root dry weight by 35 and 25%, whereas the corresponding decreases at double the recommended rates of these herbicides were 43 and 44%. Prometryn at the recommended rate did not affect root dry weight and at double the recommended rate (3.40 kg/ha) decreased it by only 12%. Averaged over the herbicide treatments Guido had significantly (148%) higher root dry weight than Rex. The herbicide \times variety interaction was significant. Guido had higher root dry weight in all the herbicide treatments. The effects of the herbicides were non-significant on Rex. In Guido, root dry weight was decreased significantly by terbutryn/terbuthylazine and bentazone at double the recommended rates of application.

Shoot dry weight

Averaged over the two varieties all the herbicides at double the recommended rate and terbutryn/terbuthylazine even at the recommended rate decreased shoot dry weight significantly (Table 4). The recommended rates of terbutryn/terbuthylazine, prometryn and bentazone decreased shoot dry weight by 23, 17 and 18%, respectively, compared to the unsprayed control. Averaged over the herbicide treatments Guido had significantly higher shoot dry weight than Rex. The herbicide \times variety interaction was significant. Shoot dry weight was not significantly affected by any of the herbicide treatments in Rex, but was decreased significantly by prometryn at double the recommended rate and by bentazone at both the rates in Guido.

Pod dry weight

All three herbicides at double the recommended rates decreased pod dry weight per plant significantly (Table 4). Guido produced significantly higher pod dry weight than Rex. The herbicide \times variety interaction was significant. There were no significant effects of herbicides on Rex. However, in Guido prometryn and bentazone at double the recommended rates significantly decreased pod dry weight.

Table 3
Effect of herbicides on nodule dry weight and root dry weight in two pea varieties

Herbicides	Rate (kg/ha)	Nodule dry wt/plant (mg)			Root dry wt/plant (g)		
		Varieties		Mean	Varieties		Mean
		Rex	Guido		Rex	Guido	
Terbutryn/terbuthylazine	1.40	105	262	184	0.64	1.65	1.14
	2.80	96	167	132	0.50	1.50	1.00
Prometryn	1.70	93	248	170	0.91	2.89	1.90
	3.40	65	160	113	0.97	2.13	1.55
Bentazone	1.44	98	161	129	0.84	1.78	1.31
	2.88	68	99	84	0.61	1.35	0.98
Unsprayed control		122	257	190	1.07	2.44	1.76
Mean		92	193		0.79	1.96	
S.E. (D.F.=26)		Herbicides (H)		17.7			0.177
		Varieties (V)		9.4			0.095
		H × V interaction		25.1			0.251

Table 4
Effect of herbicides on shoot (stem + leaf + pods, including seeds) dry weight and pod dry weight of two pea varieties

Herbicides	Rate (kg/ha)	Shoot dry wt/plant (g)			Pod dry wt/plant (g)		
		Varieties		Mean	Varieties		Mean
		Rex	Guido		Rex	Guido	
Terbutryn/terbuthylazine	1.40	10.1	20.0	15.1	5.61	10.27	7.94
	2.80	8.5	20.4	14.4	5.13	10.18	7.66
Prometryn	1.70	12.8	19.7	16.2	6.60	9.60	8.10
	3.40	13.6	17.2	15.4	7.11	7.85	7.48
Bentazone	1.44	14.8	17.1	16.0	8.51	8.94	8.72
	2.88	13.0	15.0	14.0	7.59	7.88	7.73
Unsprayed control		14.0	24.9	19.5	7.76	12.88	10.32
Mean		12.4	19.2		6.90	9.66	
S.E. (D.F.=26)		Herbicides (H)		1.22			0.798
		Varieties (V)		0.65			0.427
		H×V interaction		1.73			1.129

Discussion

Effects of varieties

Pea varieties show differential growth. In the present study the variety Guido had higher values than Rex for all the parameters. When averaged over the herbicide treatments Guido had more nodules (Table 2) and higher nodule dry weight (Table 3) compared to Rex. Large genotypic variability for number of nodules and their weight has also been reported by other authors in peas

(Singh et al., 1994), soybean (Herridge and Betts, 1988), cowpea (Zari et al., 1978), faba bean (Cordovilla et al., 1995), kidney bean (Chaverra and Graham, 1992; Hernandez et al., 1993), pigeonpea (Nambiar et al., 1988; Rupela and Johanson, 1995), groundnut (Nambiar et al., 1988) and chickpea (Nambiar et al., 1988; Sattar et al., 1995). Differences between varieties may be due to differences in the speed of nodule formation, which also varies between varieties, as reported in peas (Mårtensson and Rydberg, 1996) and kidney bean (Chaverra and Graham, 1992).

The number and size of nodules is controlled by the host (Nutman, 1981). In the present study the large-seeded variety Guido produced more (Table 2) and heavier (Table 3) nodules than the small-seeded variety Rex. Seedlings from large seeds have been shown to emerge faster and have more nodules (Cash and Ditterline, 1996). Similarly, while studying the effect of seed size and plant growth on nodulation in lima bean (*Phaseolus lunatus* L.) Dobert and Blevins (1993) found a significant positive correlation between initial seed weight, plant weight and nodule number and weight, and they were of the view that nodule formation is under the influence of seed-derived factors and the continued accumulation of nodule tissue is related to shoot growth. In Guido the greater number and dry weight of nodules may be due to the greater root growth recorded in this variety (Table 3). However, nodule dry weight per g of root dry weight was almost the same in both the varieties.

Guido had higher root (Table 3), shoot (Table 4) and pod (Table 4) dry weight. The greater number of nodules and the higher nodule and root weight in Guido probably resulted in higher aboveground plant growth in this variety, as greater nodulation should have resulted in high rates of nitrogen fixation, and better root growth can enable plants to absorb more nutrients and water for shoot growth. Alternatively the better shoot growth in Guido, compared to Rex, might have provided more photosynthates for better root and nodule growth.

In conclusion it appears that the differences observed between varieties could be due to differences in seed size as seed size can affect nodulation and plant growth as discussed earlier. Alternatively the genetic make-up (differences other than seed size) of the two varieties might also have affected nodulation and plant growth in these varieties.

Effects of herbicides

Herbicides significantly influenced the number of nodules (Table 2), nodule and root dry weight (Table 3), and shoot and pod dry weight (Table 4) and all these parameters tended to decrease at double the recommended rate of application. Despite there being no visual phytotoxicity by terbutryn/terbuthylazine and prometryn at the recommended and double the recommended rates, adverse effects on plant growth were observed. This might have happened due to the internal effects of the herbicides on growth processes

in the plants. All the herbicides tested in this study are known to adversely affect photosynthesis in plants (Worthing et al., 1982; Dodge, 1990; Tomlin, 1995). Thus, the decreases observed in the above-mentioned parameters might be the result of decreased photosynthesis. Despite no visual symptoms on the plants, decreased photosynthetic rates have also been reported to be caused in kidney bean by bentazone (Bethlenfalvay et al., 1979) and in crownvetch (*Coronilla varia*) by atrazine (Cardina et al., 1986). In plants a process can be inhibited beyond recovery even without showing any visible effects (Devine et al., 1993).

Kumar et al. (1981) also reported a decreased number of nodules and nodule weight in chickpea after the application of prometryn. Terbutryn decreased the number and weight of nodules in groundnut (van Rensburg and Strijdom, 1984) and lentil (Sandhu et al., 1991). Bentazone decreased, though non-significantly, the number and weight of nodules in kidney bean (Schnelle and Hensley, 1990), soybean (Ozair and Moshier, 1988; Ozair et al., 1990) and only the number of nodules in soybean (Yueh and Hensley, 1993) and red clover (Mårtensson, 1992). Some of these reports also recorded the effects of herbicides on root or shoot growth, which were generally adversely affected. Al-Khatib et al. (1995) also reported injury to peas and a reduction in yield with bentazone applied at 1.68 kg/ha.

In the present studies the nodule dry weight per plant in Guido was significantly correlated ($r = 0.789$, $P < 0.05$) with shoot dry weight per plant, but no correlation between these parameters was observed in Rex. The significant correlation between shoot and nodule dry weight in Guido indicates that greater nodule weights contributed positively and significantly to greater shoot growth in this variety. However, in Rex such a correlation was not observed. If the plants are grown in soil it is rather difficult to relate the effects of herbicides on nodulation to effects on plant growth because a decrease in nitrogen fixation due to the decrease caused in either nodulation or specific nitrogenase activity or both by herbicides, if any, can be compensated for by nitrogen uptake from the soil by the plants (Rennie et al., 1982; Rennie and Dubetz, 1984a; 1984b). Such effects can only be studied with certainty under conditions where the plants have biological nitrogen fixation as the only source of nitrogen for growth.

Herbicide \times variety interaction

In most cases where the herbicide \times variety interaction was significant (Tables 2–4) the effects of certain herbicides were significant in Guido but not in Rex. In the case of non-significant effects compared to their respective unsprayed controls, larger decreases in various parameters due to different herbicide treatments were observed in Guido than in Rex. Varietal differences in the responses to various herbicides have been reported in kidney bean (Bauer et al., 1995) and soybean (Osborne et al., 1995).

Generally nodulation (Tables 2 and 3) and plant growth (Tables 3 and 4) decreased with the application of high rates of herbicides in both the varieties. However, at double the recommended rate terbutryn/terbuthylazine, followed by prometryn had a less adverse effect on the number and dry weight of nodules compared to bentazone in both the varieties and the adverse effects of all the herbicides were greater in Guido than in Rex.

The higher adverse effect of bentazone on nodulation in both the varieties was mainly due to a decrease in the number of small- and large-sized nodules (Table 2). This herbicide had a severe effect on plant growth, especially in Guido, which might have resulted in a reduced supply of photosynthates to the nodules, with the result that small-sized nodules shrivelled and large nodules did not grow further. For a foliage-applied herbicide, toxicity is partly related to the amount of spray retained (Sagar et al., 1982). The greater foliage of the plants of Guido might have received and then absorbed greater amounts of this foliage-applied herbicide compared to Rex. Furthermore, there could be differences in the retention of the herbicide on the leaves and then in the metabolism in the plants of the two varieties. It is known that the selectivity of bentazone is based both on differential retention, absorption and translocation and on detoxification (Hance and Holly, 1990). Bentazone should not be applied if the temperature is higher than 21°C (BASF, 1996). Although peas tested safe according to the pea leaf wax test (Gane et al., 1984; PGRO, 1993) before the application of bentazone, adverse effects on plant growth were still observed, possibly due to the higher temperature at and after herbicide application, as higher temperatures result in greater absorption of a herbicide (Wanamarta and Penner, 1989). With foliage-applied herbicides higher temperatures increase the penetration of herbicides into the leaves, probably by altering the physical characteristics of the cuticle and the velocity of certain physiological processes (Sagar et al., 1982). Similarly, high light intensity might have stimulated the penetration of bentazone. No temperature data were recorded in the glasshouse. However, higher temperatures are expected in the glasshouse during May and early June (the plants were harvested in early June).

Prometryn caused a reduction in root growth, as also reported by Kumar et al. (1981) in chickpea, but the other two herbicides had even more adverse effects than prometryn on root dry weight. The reduction in root growth might be the result of reduced shoot growth due to the decreased photosynthesis caused by these herbicides (Worthing et al., 1982; Dodge, 1990; Tomlin, 1995), with a consequently reduced supply of carbohydrates to the roots.

There were two results that were unexpected. First, prometryn at the recommended rate (1.70 kg/ha) decreased the nodule dry weight in Rex but decreased the number of nodules in Guido. Second, prometryn and bentazone at double the recommended rates decreased the number and dry weight of nodules in the variety Rex to a greater extent compared to their effects on plant growth.

The reason for the first point could be genetic differences between the varieties. Regarding the second point, as the data were recorded 16 and 4 weeks after the application of prometryn and bentazone, respectively, these might have decreased plant growth in the early stages and then with time the effect may have disappeared. Kumar et al. (1981) also reported a greater adverse effect of prometryn on chickpea at 42 than at 56 days after sowing. As regards the decrease in nodulation observed at that stage, nodulation may also have decreased at an early stage and could have been expected to increase as the effect of the herbicide diminished with time, but this did not happen as the photosynthates were possibly used for the production and growth of pods at the expense of nodulation.

Conclusions

The results show that overall the variety Guido had better growth and higher pod weight than Rex. For the variety Rex bentazone and prometryn were better herbicides, whereas in Guido terbutryn/terbuthylazine caused comparatively less reduction in growth compared to other herbicides. All these effects were due only to the herbicides and not due to weeds as they were controlled manually as and where required. The results stress the necessity to screen a greater number of varieties for the various herbicides commonly used for controlling weeds in peas for their possible effects on the nodulation, growth and yield of this crop. Similar studies should also be done in other legumes.

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QUALITY AND YIELD IN ST. JOHN'S WORT (*HYPERICUM PERFORATUM* L.) HARVESTED IN DIFFERENT PHENOLOGICAL STAGES

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Two experiments were conducted in the province of Ñuble, Chile during the 1997/98 and 1998/99 seasons with the objective of evaluating the effect of harvesting date on the yield and quality of St. John's wort (*Hypericum perforatum* L.) in the second year of production. The apical 25 cm of the stem were harvested in the following stages: flower bud, beginning of flowering, full flower and petal drop. A randomized complete block design with four replications was used. The best yield (fresh, dry and threshing weight) and the highest hypericin content were obtained at the petal drop stage. Nevertheless, the results indicate that the best time to harvest St. John's wort is when 10 to 20% of the flowers are open and the rest are in the bud stage.

Key words: *Hypericum perforatum*, *Hypericaceae*, hypericin, yield, harvesting stage, flower, leaf

Introduction

St. John's wort (*Hypericum perforatum* L., *Hypericaceae*) is known mainly for its antidepressive, antibacterial, antiviral, scar-forming and burn-healing properties (Gaedcke, 1997; Piperopoulos et al., 1997; O'Hara et al., 1998; Berti et al., 2000; Hevia et al., 2000b). It has been noted that extracts of St. John's wort contain many compounds that are responsible for these pharmacological effects. These include the naphthodianthrone (hypericin, pseudohypericin), hyperforin, hyperoside, rutin, quercetin and biapigenin (Staffeldt et al., 1994; Piperopoulos et al., 1997; Repcák and Mártonfi, 1997). However, hypericin is utilized as the key compound in measuring the quality of St. John's wort. In some cases the levels of hypericin demanded today in the international market exceed 0.15% (Hevia et al., 2000b).

The secondary metabolites are unevenly distributed in the plant, hypericin accumulating mainly in flowers (von Hölzl and Ostrowski, 1987; Repcák and Mártonfi, 1997; Hevia, 1999). Consequently, one way of improving the quality of dehydrated botanical parts of St. John's wort would be to examine the developmental stage of the flowers at harvest.

The objective of this research was to evaluate the yield and quality (hypericin percentage) of St. John's wort harvested at different developmental stages in order to define the phenological stage at which it should be harvested to achieve a good yield of fresh material with adequate quality.

Materials and methods

Two experiments were carried out in the province of Ñuble (annual mean temp. 13.9°C; max. temp. (January) 29–32°C; min. temp. (July) 3.8°C; annual rainfall 900–1100 mm) in 1997/98 and 1998/99 using St. John's wort in the second year of production. In both experiments, the crop was watered and *Chrysolina quadrigemina* was controlled with 150 ml of Alfacypermethrin (Fastac) in 200 l ha⁻¹ of water. Weed control was conducted manually. Nitrogen was applied to the crop in the form of sodium nitrate: 50 kg per hectare in the first year, and 100 in the second year. In both trials, the plants were harvested in the same developmental stage.

The 1997/98 experiment was carried out 4 km south of Chillán Viejo (36°03' S, 72°06' W). The soil belongs to the Bulnes (Vertic Haploxeralf) series, with a silty loam texture and analysis before the trial showed pH=6.20 and 3.85% organic matter.

The 1998/99 experiment was conducted at the Experimental Station of the Agronomy Faculty of the University of Concepción, Campus Chillán (36°03' S, 72°06' W), in a soil of the Diguillín series, classification Typic Melanoxerand. The analysis of the soil showed 7.8 ppm N-NO₃, 25.1 ppm P and 590 mg K kg⁻¹ of soil.

Harvest timing

In both trials, the plants were harvested at the following developmental stages:

- Flower buds: 50% of the flower stems had at least one visible bud.
- Beginning of flowering: 50% of the flower stems had at least one flower open.
- Full bloom: 50% of the flower stems had at least two or more flowers open.
- Petal fall: when all the flower stems had all their flowers open.

Experimental design

A randomized complete block experimental design was employed, with four replications, in which the treatments corresponded to the phenological stages of the crop at the moment of harvest.

Evaluation

The following evaluations were made:

Fresh and dry matter yield per hectare. The top 25 cm of the plants were harvested manually from a linear metre of the center row of each treatment, weighed fresh, and then dried at 50°C for twelve hours in a Binder forced air oven. The dry weight yield was determined by the difference in the weight.

Threshing yield. Six 25 cm stem segments were chosen at random. They were weighed fresh and then separated into flowers, leaves and stems. These organs were weighed fresh separately and then dried in a forced air oven at 50°C for 12 hours to obtain the dry weight. These data were used to determine the threshing yield, defined as the quotient between the sum of the dry weights of the flowers and leaves, and the fresh weight of the complete stem segment harvested (flowers, leaves and stems). Using the above data the percentage of flowers, leaves and stems on a dry matter basis was also determined.

Hypericin content. This was determined for the flowers, leaves and stems separately as well as for the whole stem harvested. For this purpose, six plants from the middle row were randomly selected for harvest. The samples were dried and then ground in a Retsch mill using a 500 mesh sieve. Subsequently, the hypericin content was determined in the 1997/98 trial by means of the Southwell and Campbell (1991) method and in the 1998/99 trial by the official German method (Anonymous, 1991). In both cases the sample was continuously protected from light.

Statistical analysis

The results from both experiments were submitted separately to variance analysis (ANOVA) to determine the significance of the factors being studied. The transformation of the data was performed using the relation $(X+0.5)^{1/2}$ (Steel and Torrie, 1985). The means were compared via the LSD_{5%} test.

Results and discussion

1997/98 trial

The crop was located on the northeast side of a poplar grove that covered it with shade starting at mid-day. This situation may have had a negative influence on the development of St. John's wort, due to a reduction in the ambient temperature, a lower absorption of photons (between 600 and 700 nm), and probable water stress. For these reasons, the plants had reduced growth and produced a smaller number of umbels. In this regard, Dachler and Pelzmann (1989) indicated that St. John's wort needs sunny locations to fully develop its potential.

The time between bud formation and petal fall was longer in this trial than in the 1998/99 trial due to the lower absorption of solar radiation, generally between 600 and 700 nm (Larcher, 1994). As a result, the photosynthetic rate and accumulation of photosynthates decreased, retarding the development of the crop.

1998/99 trial

The crop was affected by an attack of *Rhizoctonia solani*, which destroyed the plants. For this reason, it was not possible to carry out a second cut in the season.

The foreign literature also mentions the attack of numerous fungi that may arise either in the seedling stage or during the vegetative period of the plant in the first or even subsequent years. These are *Fusarium*, *Alternaria*, *Phoma*, *Pythium*, *Sclerotinia*, *Verticillium*, *Rhizoctonia* and *Colletotrichum* (Bomme, 1997). Among the diseases that affect this species in Chile, the fungi *Rhizoctonia solani* and *Colletotrichum gloesporioides* have been detected (Berti et al., 2000).

Fresh matter yield

In Figure 1 it can be observed that the greatest yield of fresh matter per hectare in both trials was obtained when the crop was harvested at the petal fall stage, reaching 3796 and 5846 kg ha⁻¹ in 1997/98 and 1998/99, respectively. These values were much lower than those obtained by Dachler and Pelzmann (1989), who reported yields of fresh matter reaching 20,000 kg ha⁻¹, without indicating the stem length harvested.

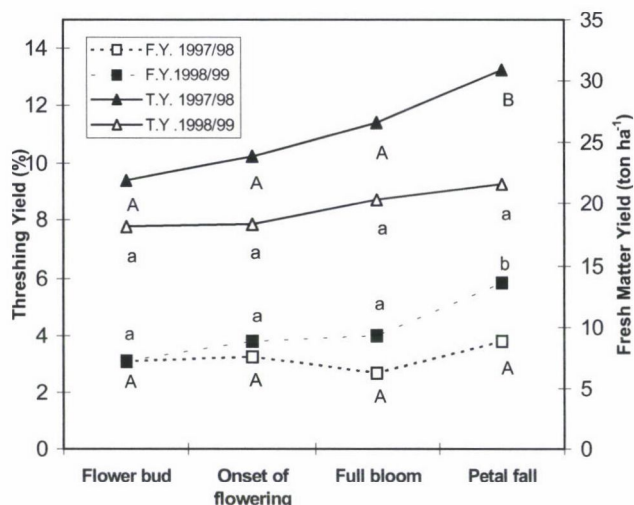


Fig. 1. Threshing yield (T.Y.) and fresh matter yield (F.Y.) related to harvesting stage. Different letters for the phenological stages of the same year indicate significant differences ($P \leq 0.05$) according to the LSD test.

This difference can be attributed, in part, to the genotypes of the plants, the location, the cut length and diseases (Bomme, 1997; Büter et al., 1998). The plants utilized in this study were obtained from seeds harvested from wild Chilean plants. In experiments conducted in Chillán with plants obtained from Chilean seed and seed imported from Germany, the yields of fresh and dry matter were always greater in plants of German origin, due to the latter having a greater development of stems, foliage and flower buds (Hevia et al., 2000a).

The lower yield observed in the 1997/98 experiment could be attributed to the shade permanently covering the crop, as mentioned previously, the type of soil and probably water stress.

According to the variance analysis there were no significant differences ($P \leq 0.05$) between the treatments in the same trial. From the data, it follows that as the crop advanced in its developmental stage towards physiological maturity, the phytomass accumulated tended to increase ($P > 0.05$). This tendency can be explained by the fact that *St. John's wort* is a long-day plant, which in the winter months only grows as a rosette. As a consequence, the stimulation of long days and warmer night-time temperatures in the spring initiate the lengthening of the stems, producing an increase in the leaf area index as well as the accumulation of hypericin. The increase in leaf area index favours solar radiation absorption and a greater photosynthetic rate. Thus, a greater accumulation of photosynthates occurs, these being later transferred from the leaves to the reproductive parts of the plant, favouring the greater production of phytomass (Salisbury and Ross, 1994).

The dry matter yield followed a similar trend to that observed for the fresh matter yield. The dry yields reached in this study fluctuated between 760 and 1690 kg ha⁻¹. These values might have been higher if it had not been for the shading in the 1997/98 trial and the attack of *Rhizoctonia solani* in the 1998/99 trial, as noted previously.

Threshing yield

Both experiments exhibited similar behaviour (Fig. 1). The threshing yield tended to increase ($P>0.05$) when the harvest was delayed. This was probably due to the accumulation of dry matter for a longer period of time, together with a reduction in the humidity, without increasing the dry weight of the stems. In this connection, Libbert (1993) mentions that the growth of the stems stops at the beginning of anthesis.

Relative percentage of flowers, leaves and stems

Figure 2 shows the relative percentages of flowers, leaves and stems on the harvested stem segments in the 1997/98 and 1998/99 trials. A similar trend was observed in both years for the flowers and leaves, with the highest relative flower ratio at the end of anthesis (petal drop) and the highest relative leaf ratio at the bud stage. For the stems the highest ratio was found at the full flower stage in the first trial and at petal drop in the second. The latter was associated with a smaller relative proportion of leaves.

The results indicate that in the 1997/98 trial the ratio of flowers and stems was lower and the proportion of leaves greater than in the 1998/99 trial. This could be explained by the shade that covered the crop in the first trial and the attack of *Rhizoctonia solani* in the second trial, which caused numerous plant stems to dry.

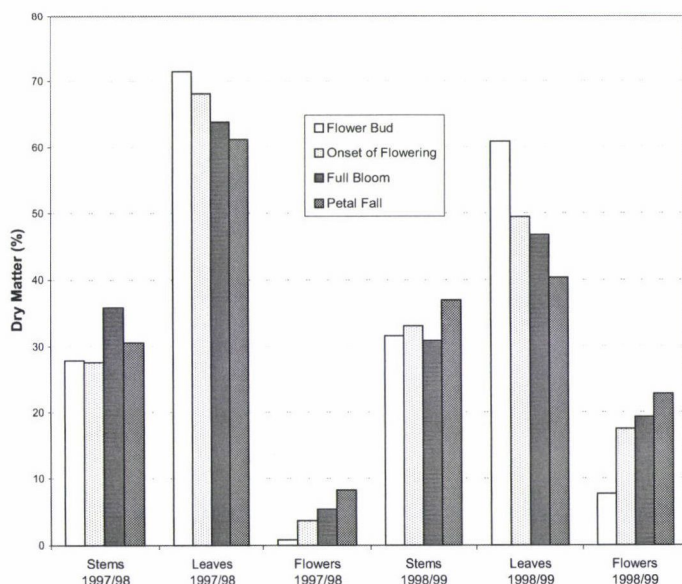


Fig. 2. Stem, leaf and flower ratio in stem segments harvested in 1997/98 and 1998/99 related to harvesting stage.

Hypericin content

The hypericin content (Fig. 3) was always higher in the flowers than in the leaves, and this in turn was always superior to that of the stems, exhibiting an approximate ratio of 30:10:1. Similar proportions were also reported by Southwell and Campbell (1991) in Australia and Staffeldt et al. (1994) in Germany, but the present results were, in general, 25% lower than the contents reported by these authors. The difference could be attributed to genetic or environmental characteristics, or both (Debrunner and Simonnet, 1998). The final composition is also influenced by analytical factors, like the particle size of the sample, the analytical method, and light exposure, which is known to enhance the conversion of protohypericin and protopseudohypericin to hypericin and pseudohypericin, thereby increasing the values (Southwell and Campbell, 1991; Gaedcke, 1997).

In the 1997/98 experiment, as the plants approached physiological maturity the hypericin content diminished in the flowers, while that of the leaves and stems remained relatively constant (Fig. 3).

In the laboratory it was observed that during the drying and preparation of the samples the anthers fell apart very easily. Since these are the floral organ with the greatest content of the secondary metabolite, being approximately 115 times superior to the entire flower, this explains the tendency for the hypericin content of the flowers to decrease as the plants advance toward the full bloom stage.

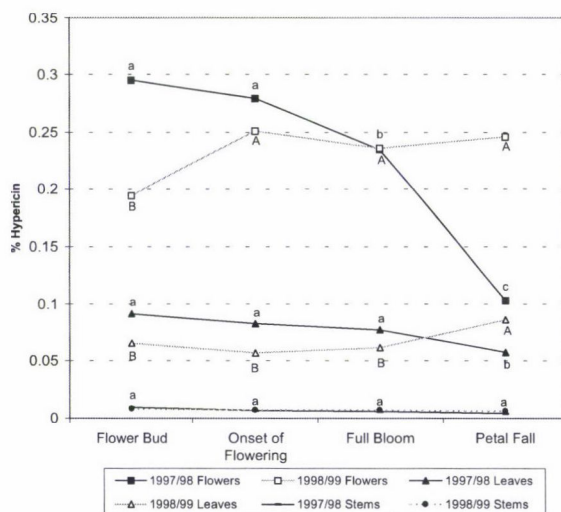


Fig. 3. Hypericin content in flowers, leaves and stems in the 1997/98 and 1998/99 trials, related to harvesting stage. Different letters for phenological stages in the same year indicate significant differences ($P \leq 0.05$) according to the LSD test.

To verify whether this reduction was effectively due to the loss of anthers, the 1998/99 trial was conducted taking care that the anthers did not fall apart during the drying and preparation of the samples.

The hypericin content of the flowers was higher when the crop was harvested at the beginning of flowering (0.250%). In later harvests, at full flowering and petal fall, the values were 0.235 and 0.246%, respectively (Fig. 3). Nevertheless, there was no significant difference between these three stages of development. In the flower bud stage a lower hypericin content, 0.194%, was found in the flowers ($P \leq 0.05$).

The flower hypericin content increased ($P \leq 0.05$) as there was a larger number of open flowers. Although the correlation coefficient was significant ($r=0.59^*$), an apparently contradictory situation was encountered. At the beginning of flowering, a greater hypericin content was found in the flowers, but with a smaller relative percentage of open flowers than that found in the two later stages (Figs. 2 and 3). This is probably because the flowers were not completely open at the beginning of flowering, and therefore, a smaller number of anthers were lost during harvesting and drying (Hevia et al., 2000a).

In the leaves, the hypericin concentration of both samples fluctuated between 0.06 and 0.09% (Fig. 3), not reaching the 0.10% currently demanded by buyers. There was no difference between the first three harvest dates in either experiment, although they were all different from the last harvest date. It should be noted that, while in the 1997/98 trial the hypericin content was lowest at petal fall, in the 1998/99 trial the largest concentration of the metabolite was observed at this stage.

Possibly, the increase in the hypericin content in the leaves could have been a consequence of stress, because the plants were affected by a *Rhizoctonia solani* attack and lack of water, which probably increased the number of black glandular dots in the leaves. In this regard Gray et al. (1998) indicated that when plants of St. John's wort were submitted to water stress the number of oil glands increased in the leaves and flowers, and thus, the hypericin content of both structures increased.

The hypericin content in the stems was always very low and no significant differences were detected between the stages at which the plant was harvested in either experiment (Fig. 3).

On comparing the hypericin content in the organs that compose the floral stem, i.e. in the flowers, leaves and stems, it can be concluded that the hypericin content (dry basis) in the stems was lowest and fluctuated between 0.007 and 0.008%. In the leaves it was somewhat higher, varying between 0.056 and 0.086%, while in the flowers higher values were observed, between 0.19 and 0.25%. The ratio between the average values for flowers, leaves and stems was 31:10:1, coinciding with the results reported by Southwell and Campbell (1991), Staffeldt et al. (1994) and Hevia (1999). This indicates that in spite of the low proportion made up by flowers in the stem segment weight, they make an important contribution to the total hypericin content of the segment harvested.

In the 1998/99 experiment, the hypericin content of the harvested stem segment was analytically determined (Fig. 4). The highest metabolite accumulation was observed at petal fall (0.104%), followed by the onset of flowering stage (0.81%) and finally full bloom (0.074%). The lowest value was found at the flower bud stage.

The high hypericin content obtained at the petal drop stage – in the complete stem segment harvested – was due to the larger quantity of flowers (Fig. 2). This was demonstrated by the positive correlation ($r=0.74^*$) between the quantity of flowers (as a dry weight percentage) and the hypericin content of the harvested stem segment.

However, it cannot be ignored that at petal drop the relative proportion of the stem was greater ($P \leq 0.05$) than at other stages of development. This might have caused a dilution effect in the hypericin content of the harvested stem segment (Hevia et al., 2000a). Therefore, the greater hypericin content observed might be a consequence of water stress (Gray et al., 1998) and *Rhizoctonia solani*, which also caused stress to the plant, as indicated above. Possibly for this reason a process of protection could be triggered in the leaves and flowers, increasing the hypericin concentration at the same time as the cellular volume decreased, with a consequent reduction in turgidity (Sánchez and Aguirreolea, 1996).

In the 1997/98 experiment, the hypericin content was not directly determined in the harvested flowering shoot, but by estimating its values based on the percentages in the flowers, leaves and stems in the harvested segment (Fig. 4). Accordingly, the hypericin content in the harvested segment diminished as the number of open flowers increased, therefore explaining the larger concentration at the onset of flowering. These results give a good reflection of what happens in practice, since neither in the field nor on the dehydrated plants is it possible to avoid the falling apart of the anthers.

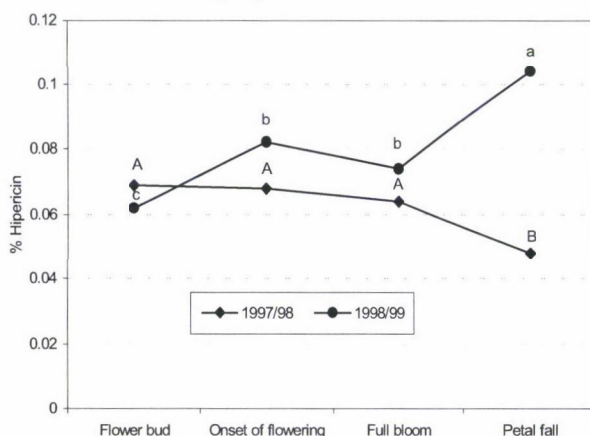


Fig. 4. Hypericin content of the harvested stem, related to harvesting stage. Different letters for phenological stages in the same year indicate significant differences ($P \leq 0.05$) according to the LSD test.

These results indicated that the harvest of St. John's wort should be conducted when no more than 10-20% of the flowers are open and the rest still remain in the flower bud stage, thus avoiding the loss of the anthers.

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EFFECT OF SEED TREATMENT ON THE EMERGENCE OF INBRED LINES OF MAIZE (*ZEA MAYS* L.)

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The emergence time and emergence percentage of four inbred lines with different degrees of chilling tolerance (Mo 17, HMv 5316, HMv 5301, HMv 5478) and the dry mass of young shoots were examined in a seed dressing experiment carried out in pots and sown early under field conditions, on soil infected with pathogens or free of infection (control).

The advantage of seed dressing was manifested for all the traits examined, with the exception of emergence time, and the dressing agents were found to exhibit a certain degree of variety specificity. The time to emergence was determined chiefly by the genotype of the inbred lines.

Differences were observed when the inbred lines were ranked on the basis of the emergence time and the emergence percentage. This was in agreement with the fact that no correlation was observed between the emergence percentage and the emergence time.

Genotype HMv 5316 proved to be best on the basis of emergence time and HMv 5301 for emergence percentage. The poorest results for all the traits examined were recorded for Mo 17, which is in agreement with the poor chilling tolerance of this inbred line.

In summary, the following conclusions can be drawn: if the chilling tolerance and growth vigour of the genotypes are to be satisfactorily described, traits characteristic of initial plant development (dry mass of the shoots, size of the leaf area, etc.) should also be considered in addition to the emergence time and emergence percentage.

Key words: maize seed dressing, emergence time, emergence percentage, shoot dry mass, inbred line

Introduction

In Hungary and in countries with a cooler climate, the chilling tolerance of hybrids and inbred lines in early spring is of special importance, as demonstrated by the fact that numerous authors have dealt with this property of maize.

The first experiments on the chilling tolerance of maize were reported by Tatum (1942), Tatum and Zuber (1943), Neal (1950), Neptune (1953) and Clark (1954). When testing chilling tolerance, breeders and geneticists generally used a combination of two temperatures, cold incubation and optimum germination temperature. These methods were only able to provide valuable information on the chilling tolerance of maize at germination (Marton, 1990a; Marton et al., 1997).

The results of the cold test depend greatly on other external factors such as seed dressing, the length of the cold treatment, the temperature applied and the pathogenic conditions of the germination medium. With an increase in the length of the cold incubation phase there is a decline in the germination

percentage even in sterile medium. This reduction in the germination ability is even more pronounced in the field due to the presence of pathogens. In addition to the influence of environmental factors on the cold test results, Wortman (1950), Rinke (1954) and Andrew (1954) also proved the role of hereditary factors. Neptune (1953) established the fact that the cold test value declined with a rise in the level of inbreeding, while the difference between the lines increased.

According to the results of Szundy and Kovács (1981a, b) and Szundy and Marton (1999), the chilling tolerance of populations with different levels of heterozygosity and of their hybrids is in positive correlation with the level of heterozygosity of the female parent.

Marton (1991; 1997a) considered a temperature range of 7–14°C to be satisfactory for measuring the chilling tolerance of the genotypes and investigating their growth at low temperature. This temperature range can be successfully applied in the evaluation and comparison of inbred lines for chilling tolerance.

Herczegh (1978) elaborated a modified cold test in which a 10-day cold treatment at 8°C was followed by emergence at a temperature far below the optimum (13.5°C). In addition to the emergence percentage, the genotypes were also evaluated on the basis of emergence rate and a cold tolerance index elaborated by Herczegh. After examining a large number of basic breeding stocks, it was found that, on the basis of the number of days to emergence, lines of the Lancaster (A) type emerged three days later than those of the Reid Yellow Dent (B) type. Stocks of European origin resembled Reid Yellow Dent in this respect. Hybrids between European and Reid Yellow Dent lines had better chilling tolerance than A×B hybrids. This was of special importance, because the yield performance of these hybrids was also better than that of A×B hybrids.

Herczegh and Marton (1986) investigated the germination heat minimum of hybrids and inbred lines in a temperature gradient chamber. The results confirmed that the temperature requirements of the various genotypes differ considerably. Stamp (1984) also found great differences between the temperature requirements of inbred lines, which could cause problems especially in seed production in synchronising the flowering of parents with different degrees of earliness. These results serve to emphasise the fact that the temperature minimum required for emergence should be determined for each genotype separately; it is impossible to give a general temperature threshold value for maize.

Almost eighty years ago Dickson (1923) published information on the importance of pathogens when maize was germinated in the cold. The role of these pathogens was demonstrated by Hoppe and Middleton (1950) as the difference between cold test values in the field and when the soil was sterilised. After artificial inoculation with *Fusarium* species, Marton et al. (1990; 2000) found that the reduction in the chilling tolerance of hybrids was greatest after infection with *F. culmorum* and *F. graminearum*. Hybrids generally had greater tolerance of pathogens than inbred lines.

In cold tests carried out on undressed seeds, substantial differences may be observed in the results, depending on the pathogens present in the soil. According to Marton et al. (1988), the emergence percentage depends decisively on the resistance of the genotypes to pathogens in heavily infected soil, while in only slightly infected or pathogen-free soil the genetic chilling tolerance of the variety is decisive. In genetic studies on chilling tolerance, the effect of cold and of pathogens must therefore be distinguished. In order to avoid problems caused by pathogens several authors (Kovács, 1961; Bocsi, 1988; Maryam and Jones, 1983; Christeller, 1984; Marton, 1997b) used sterilised soil or artificial media for the genetic analysis of chilling tolerance.

Seed dressing had a significant positive effect on the emergence parameters of the hybrids and inbred lines examined, to an extent depending on the dressing agent and genotype. This was particularly true in the case of early sowing (Záborszky and Berzy, 1999).

According to Pozzi et al. (1985) the chilling tolerance of the genotypes should be studied not only in terms of emergence percentage and emergence index, but also on the basis of the fresh and dry weight of the seedlings. If the early growth period is to be exploited, chilling-tolerant maize genotypes should have not only good germination and emergence, but also satisfactory growth vigour (Mock and Eberhardt, 1972). Since the results of research on maize chilling tolerance make it quite clear that chilling tolerance at emergence and at the young plant stage are regulated by different genes (Marton, 1990b; Zametra and Cuany, 1982; Marton and Szundy, 1997), it is also extremely important to examine the growth of the seedlings after emergence.

In the light of the above, it was deemed necessary to examine a number of inbred lines in the field, under non-controlled conditions, using early sowing in order to evaluate the response of the seeds and young plants to natural cold effects on the basis of germination, emergence and initial shoot growth.

Materials and methods

Four inbred lines with different levels of chilling tolerance (Mo 17, HMv 5316, HMv 5301 and HMv 5478) were studied in the experiments. The seeds of these lines were treated with three different dressing agents: TMTD WP, Carboxin + TMTD, TMTD FS, with an untreated control.

The seeds were sown in two types of soil: 1. infected maize soil; 2. heat-sterilised soil (control). The effect of soil-borne pathogens was intensified by culturing ground maize kernels infected with fusarium ear rot on nutrient medium for ten days and then using an aqueous suspension of the mould colonies to water the seeds sown in non-sterilised maize soil. The two types of soil were placed in wooden boxes measuring 40 × 30 × 10 cm. Each box was divided into ten plots, each containing ten plants. Two replications of all four seed dressing treatments for each of the inbred lines were thus contained in each box. All the seed were sown on April 17th.

Prior to sowing, the soil was irrigated to 70% of water capacity. The seeds were covered with 5 cm soil, with a 1 cm sand layer on top to prevent soil cracking. In order to avoid water loss, the boxes were covered with polythene until emergence, after which the water deficiency was replaced every other day after weighing the boxes.

The following characters were examined:

1.) Emergence percentage: emerged plants as a percentage of sown seeds.

2.) Number of days to emergence: scored daily for each plant.

3.) Dry shoot mass (g): mass of the plants after drying at 100°C for 48 h.

The dry shoot mass was determined at the end of the experiment, 33 days after sowing.

The data were evaluated using analysis of variance according to Sváb (1981).

Results

Emergence date

Of the three factors tested, the number of days to emergence was only influenced by the genotype (Table 1). None of the interactions caused a significant modification of the emergence time.

Based on the mean emergence time data of the varieties (Fig. 1), inbred line HMv 5316 required the shortest time for emergence, suggesting that the chilling tolerance of this line was also manifested in the emergence date. The other lines all emerged at approximately the same time.

Seed dressing and soil treatments in themselves did not influence the length of the emergence time (Tables 2 and 3), since seed dressing did not significantly reduce the emergence time on either of the experimental soils. This is confirmed by the data in Figure 2, which illustrates that the various seed dressing treatments did not have a consistent role in determining the emergence date of the inbred lines.

In the case of Mo 17 a longer emergence time was recorded for dressed seeds (Fig. 2), since the treatment allowed plants to emerge which were destroyed in the control plots (Fig. 6). The protracted emergence of these plants explains the longer emergence time.

Table 1

Effect of genotype on the characters examined, averaged over seed dressing and soil

Genotype	Emergence time (days)	Emergence percentage	Dry shoot mass (mg/plant)
Mo 17	13.61	56.3	0.09
HMv 5316	12.37	81.9	0.16
HMv 5301	13.91	90.6	0.19
HMv 5478	13.65	82.5	0.16
LSD _{5%}	0.42	10.5	0.03

Table 2

Effect of soils on the characters examined, averaged over inbred lines and seed dressing

Soil	Emergence time (days)	Emergence percentage	Dry shoot mass (mg/plant)
Control	13.41	80.3	0.16
Infected	13.51	75.3	0.14
LSD _{5%}	0.30	7.4	0.02

Table 3

Effect of seed dressing on the characters examined, averaged over inbred lines and soil

Dressing agent	Emergence time (days)	Emergence percentage	Dry shoot mass (mg/plant)
Carboxin+TMTD	13.59	76.3	0.15
TMTD WP	13.49	80.0	0.15
TMTD FS	13.38	85.6	0.19
Control	13.38	69.4	0.11
LSD _{5%}	0.42	10.5	0.03

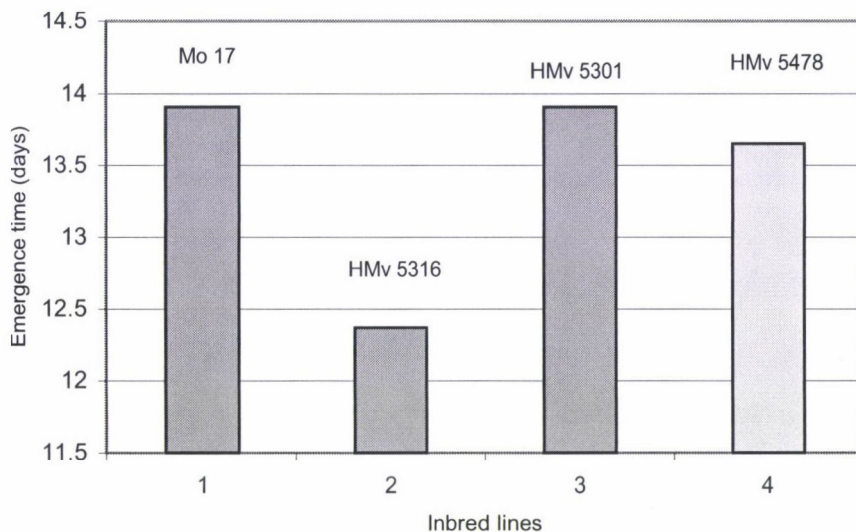


Fig. 1. Emergence percentage of inbred lines averaged over seed dressing and soil treatments (LSD_{5%} = 0.42)

Emergence percentage

As in the case of emergence time, the percentage of emerged plants depended to the greatest extent on the genotype, but seed dressing also had a substantial effect on the emergence percentage. Among the interactions, the variety \times soil interaction had a significant effect on the emergence figures, demonstrating the sensitivity of inbred lines to infected soil.

An analysis of the effect of genotype on the characters tested shows that the order of the inbred lines was not the same for emergence time and emergence percentage (Table 1). While HMv 5316 appeared to be the most chilling tolerant on the basis of emergence time, HMv 5301 gave the best results on the basis of emergence percentage. Similar results were obtained by Stamp (1984), Herczegh and Marton (1986) and Marton (1990a), confirming the finding that the correlation between the emergence dates and emergence percentages of the genotypes is not significant.

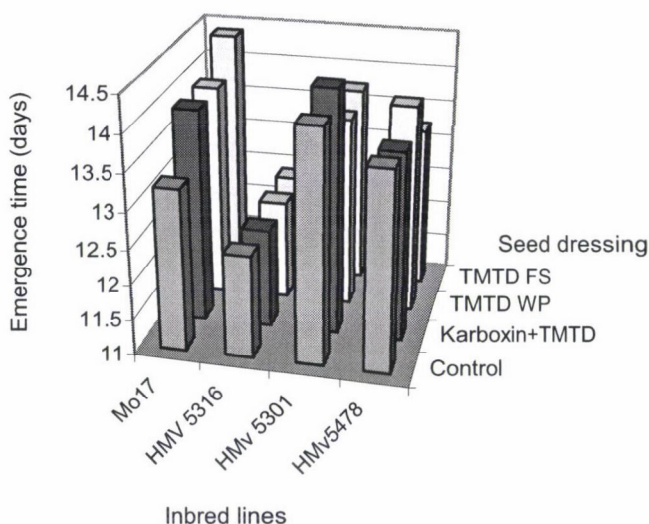


Fig. 2. Effect of seed dressing on the emergence time of inbred lines ($LSD_{5\%} = 0.84$)

On the basis of percentage emergence (Fig. 3), the emergence values of HMv 5478 and HMv 5316 were 8–9% lower compared with the best line, HMv 5301, while by far the worst emergence was exhibited by Mo 17, which had a percentage considerably lower than all the other three lines.

In contrast to the emergence date, the emergence percentage was significantly increased by the seed treatments compared to the undressed control (Table 2). Among the dressing agents, the best results were obtained with TMTD FS, followed by TMTD WP and Carboxin + TMTD. The difference between the dressing agents, however, was not statistically significant.

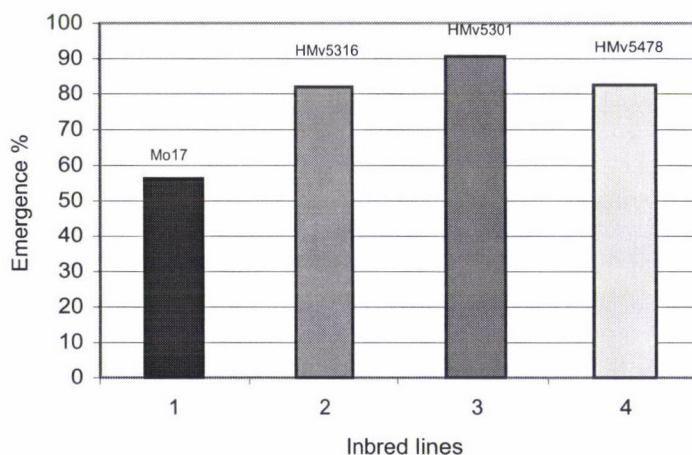


Fig. 3. Emergence percentage of inbred lines averaged over seed dressing and soil treatments ($LSD_{5\%} = 10.52$)

When the direct effect of the soil treatments was evaluated, as in the case of the emergence date, no significant difference could be demonstrated between the infected and control soils on the basis of emergence percentage, though the sterilised, control soil always gave better results numerically than the artificially inoculated maize soil (Table 2).

The percentage emergence of the lines in the various soil treatments (Fig. 4) differed depending on the natural resistance of the seed of the inbred lines to pathogens. Although the emergence percentage of Mo 17 was the lowest in infected soil, the emergence percentage of HMv 5478 dropped to the greatest extent compared with the control, indicating the great sensitivity of HMv 5478 to soil-borne pathogens. For the other two lines no substantial difference was recorded between the two soil treatments, suggesting that these lines have greater resistance to pathogens.

When the effect of seed treatments was examined in the different soils (Fig. 5), seed dressing, which did not have a significant effect on emergence time, was found to result in a clear increase in the emergence percentage in both soils. In the control soil the best results were obtained with Carboxin + TMTD, while on artificially inoculated soil seed dressing with TMTD FS gave the highest number of emerged plants compared with the undressed control.

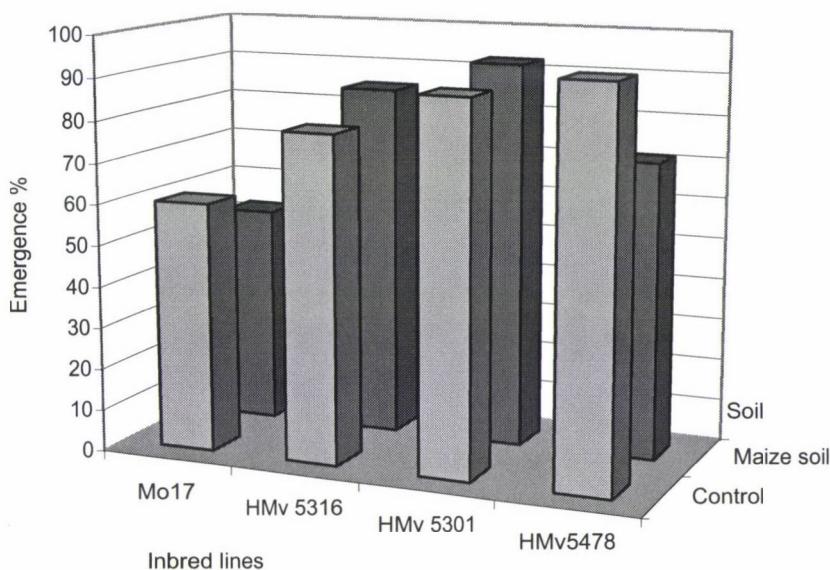


Fig. 4. Effect of soil on the emergence percentage of inbred lines ($LSD_{5\%} = 14.87$)

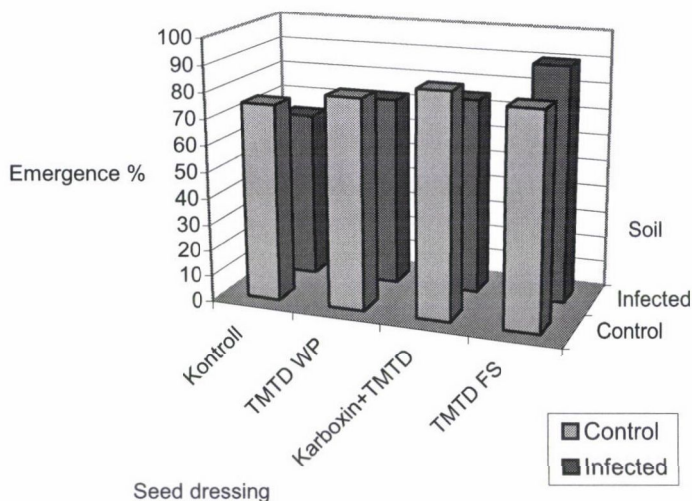


Fig. 5. Effect of seed dressing and soil on emergence percentage ($LSD_{5\%} = 14.87$)

When the effect of the seed dressing treatments was examined for the different genotypes, it was observed that undressed seeds of Mo 17 had an extremely low emergence percentage, and although this was somewhat improved by seed dressing, the number of plants emerging was still less than in lines with better chilling tolerance (Fig. 6). In treatments without seed dressing line HMv 5301 gave the best results and only dressing with TMTD WP gave a slight improvement compared with the control. The effectiveness of seed dressing was greatest in the case of line HMv 5478, where the treatments led to a pronounced improvement in emergence, the best results being obtained with TMTD FS. As previously observed in experiments on hybrids (Záborszky et al., 2001), the effect of seed dressing agents was found to be variety-specific, i.e. the efficiency of the dressing agents depended on the genotype of the inbred line.

Shoot dry mass

The dry mass of the shoots is an important parameter in characterising the chilling tolerance of young plants of inbred lines after germination. The results were evaluated on the basis of the dry shoot mass per plant.

Among the factors tested the size of the dry matter production was influenced to the greatest extent by the genotype, followed by seed dressing and soil. Among the interactions, that between variety and seed dressing was the strongest.

When the shoot dry mass of the various inbred lines was analysed, a similar tendency was found as in the case of emergence percentage. In both cases the values recorded for Mo 17 were the lowest, followed by HMv 5478. The greatest shoot dry mass was observed for seedlings of HMv 5301 (Table 1).

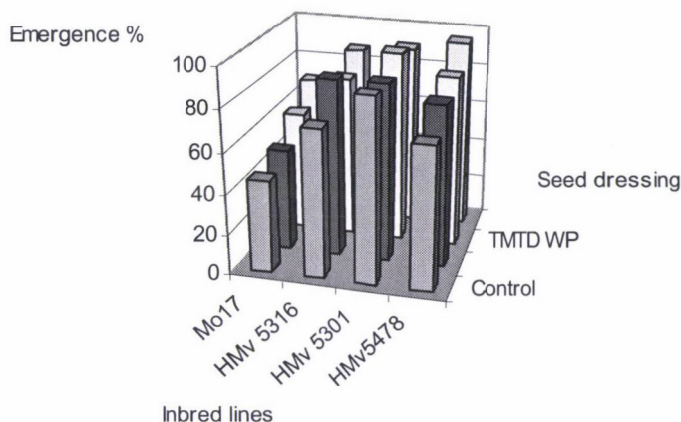


Fig. 6. Effect of seed dressing on the emergence percentage of inbred lines ($LSD_{5\%} = 21.04$)

Of the soil treatments (Table 2), the pathogen-free control soil proved to have a significantly better effect on shoot dry matter than the artificially inoculated maize soil. It was also clear that seed dressing led to an increase in the shoot dry mass of inbred lines. As in the case of emergence percentage, the effect of each dressing agent depended on the inbred line. TMTD FS proved to have the best effect in lines Mo 17 and HMv 5478, while Carboxin + TMTD was more efficient for HMv 5316 and TMTD WP in the case of HMv 5301 (Fig. 7).

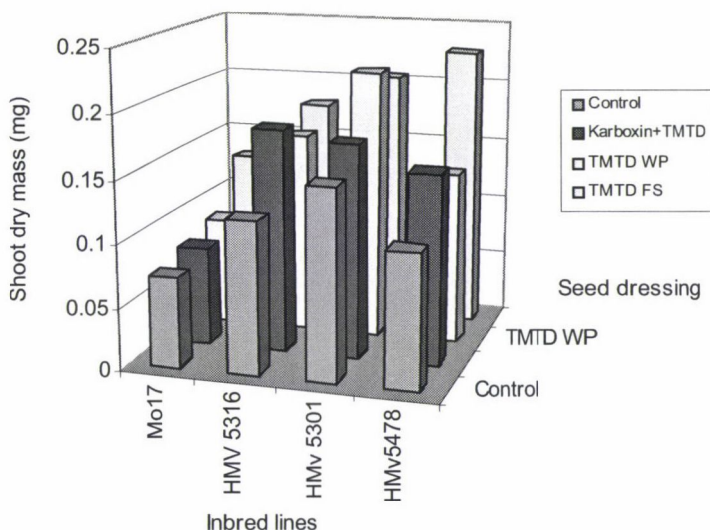


Fig. 7. Effect of seed dressing on the shoot dry mass of inbred lines ($LSD_{5\%} = 0.05$)

In summary, it can be concluded that when inbred lines were sown early, seed dressing had a significant positive effect on both the percentage emergence and the initial growth and development of the plants. As in previous studies on hybrids, the efficiency of the seed dressing agents was also found to exhibit a certain extent of variety specificity in inbred lines, which should be taken into consideration when elaborating seed production technologies.

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ZINC, LEAD, CADMIUM AND COPPER CONCENTRATIONS OF MEADOW PLANTS ALONG THE M3 MOTORWAY

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The toxic heavy metal concentrations were investigated in plants in a meadow located 51 kilometres from Budapest along the M3 motorway. The field is regularly harvested, and the hay is used as fodder. The area under investigation is situated directly alongside the protecting fence of the motorway. The soil of the area is classified as chernozem brown forest soil. In 2000 approximately 22,860 cars per month travelled on the motorway.

The plant samples were taken at a distance of 5, 10, 25, 50 and 100 metres from the motorway. At each distance 10 samples were collected. After digestion with hydrogen peroxide and nitric acid the zinc, lead, cadmium and copper contents of the samples were analysed using an ICP spectrophotometer.

After analysing the data it was established that for each of the heavy metals the concentration of metal in the plant samples decreased as the distance from the motorway grew. The measured zinc, cadmium and copper concentrations were similar to those reported by other authors (on average 44.9, 0.276 and 5.25 mg kg⁻¹), while the values of the lead concentration were lower than those published previously (on average 2.93 mg kg⁻¹). This may have been due to the widespread use of lead-free fuel.

Keywords: zinc, lead, cadmium, copper, microelement concentration, meadow plants

Introduction

Due to the increase in vehicular traffic and the denser network of roads, vehicles contaminate the environment with a substantial amount of gaseous and solid pollutants, which settle on the surface of soils and plants. In Hungary most of the agricultural land is located along motorways or main roads with heavy traffic. Therefore, research dealing with the toxic heavy metal concentrations of soils and plants on agricultural areas is also important from the point of view of public health.

Different heavy metals are emitted by vehicles in various amounts, but at a significant rate. Research carried out by Lisk (1972) demonstrated that 80% of the lead concentration in the air was released by the exhaust gas of vehicles and was deposited on roadside areas. De Luca d'Alessandro et al. (1992) also came to the conclusion that the lead compounds added to fuel were the source of 80–90 % of the total amount of lead released into the environment. According to Kádár (1995) and Schöller et al. (1991) lead comes mainly from petrol, while zinc and copper originate from wear on brake pads and other parts, and

cadmium from the mouldering of rubber and from wear on certain metal parts. The corrosion of metal parts produces chromium, nickel and copper. In Hungary Kádár (1991, 1995) and Köles et al. (1997) measured heavy metal concentrations in roadside vegetation and found that there was a positive relationship between the level of traffic and the environmental contamination. Pollutants released by vehicular traffic contaminate a broad zone along roads (Förstner, 1991; Huber, 1997; Köles, 1996; Harrison and Johnson, 1985). The considerable heavy metal contamination of the soil is not necessarily reflected in the heavy metal concentrations of the stem and leaves of plants (Keller, 1970; Graber, 1970). The amount of pollutants settling on the surface of plants may be significant; the rate mainly depends on the morphological characteristics of the plants. More lead is usually bound on rough, pubescent plants than on plants with smoother surfaces (Zuber et al., 1970). The amount of pollutants settling on the surface of plants is largely dependent on the length of the period without rain before sampling. Heavy metals in the tissues or on the surface of plants may enter the foodchain and thus the human body (Köles et al., 2001).

When data on the rate of pollution and the effects of toxic lead on living organisms were revealed, the development of lead-free fuel began. In Hungary, 95-octane, lead-free fuel was introduced in 1996, and resulted in significant changes.

The lead contamination of the environment has been reduced by the use of lead-free fuels. During the present research, changes in the concentrations of the four heavy metals (zinc, lead, cadmium and copper) released by vehicular traffic were examined in plants in a field located along a motorway with heavy traffic.

Materials and methods

The sampling place was a two-hectare field situated 51 kilometres from Budapest along the M3 motorway. The field is regularly harvested, and the hay is used for feeding cattle. The characteristic species are: *Arrhenatherum elatius* L., *Anthoxanthum odoratum* L., *Bromus erectus* L., *Bromus mollis* L. and *Agropyron repens* L. Chernozem brown forest soil is dominant on the area. The plant stand starts at a distance of 5 metres from the motorway, where there is a protective fence. Between the motorway and the fence there is a drainage ditch. This ditch prevents pollutants washed off the road by rainwater from getting onto the area under cultivation. Therefore, the heavy metal concentrations of the soil are increased mainly by deposition from the atmosphere. In 2000 approximately 22,860 cars per month travelled on the motorway. The section of the motorway under investigation was opened in 1980.

The samples were taken on 16 June 2000 after a three-week period without rain. The plant samples were taken at a distance of 5, 10, 25, 50 and 100 metres from the motorway. At each distance 10 samples were collected; each sample was taken from an area of about 5 dm².

The prevailing wind direction of the area is N-NW, so the samples were taken on the southern side of the road. The plant stems were cut 2 centimetres from the soil surface, as recommended to Albasel and Cottenie (1985) and Köles et al. (1997), and after drying (3 days, 70°C) the samples were digested by nitric acid and hydrogen peroxide. The zinc, lead, cadmium and copper contents of the samples were measured by ICP analysis. There were no industrial or agricultural activities emitting heavy metals near the sampling place.

Results and discussion

An evaluation of the concentrations found in the samples indicated that for all four elements the heavy metal concentrations of the meadow plants decreased with the distance (5–100 metres) from the M3 motorway. Table 1 shows the concentrations of heavy metal recorded and the standard deviations (SD). It can be seen that the zinc content decreased at different rates with the distance from the motorway. There was a significant difference between the concentrations measured at a distance of 5 and 100 metres ($p < 0.01$). At a distance of 25 metres the element concentration was higher than at a distance of 10 metres. Presumably a deposition zone was formed between 25 and 50 metres from the motorway due to the turbulence caused by vehicles. A similar tendency was observed for the horizontal changes in concentration of the other heavy metals under examination. These values correspond to or are very similar to those published by other authors (Table 2). It should be taken into consideration that in the present examination the samples nearest to the road were taken at a distance of 5 metres. There may be large differences between the heavy metal concentrations of samples taken directly at the roadside and from a distance of several metres. However, the present work was concentrated on plants used for feeding animals.

The copper concentration of the plants also showed a decrease as a function of the distance (Table 1). There was a significant difference between the concentrations measured at a distance of 5 and 100 metres ($p < 0.1$). Similarly to the other heavy metals, the copper content of the plants increased at a distance of 50 metres from the motorway. This might result from the formation of a deposition zone due to the turbulence conditions, as mentioned above. The present values show the greatest similarity to those of Kádár (1995) (Table 2).

The lead content of the meadow plants also decreased with distance (Table 1). There was a significant difference between the concentrations measured at a distance of 5 and 100 metres ($p < 0.1$). Comparing the present data with those of other Hungarian authors (Kádár, 1995; Póti et al., 1997) it can be seen that the lead concentrations are much lower (Table 2). This can be explained partly by the fact that other authors examined different species with different sampling methods in different phenological phases, but it may also be related to the introduction of lead-free fuel, which has led to a significant decrease in the amount of lead emitted by vehicles into the environment. As a consequence of this, the amount of lead settling on plants from the air also decreases, while the concentrations of other heavy metals, such as platinum (Wäber et al., 1996), increase due to the use of catalyzers. At a distance of 50 metres from the motorway the lead concentration of the plants was higher, as in the case of the other heavy metals investigated.

Table 1

Heavy metal concentration of meadow plants along the M3 motorway (mg kg^{-1} , dry matter) and the values of standard deviation

Our data (2000)	Zn	SD (Zn)	Cu	SD (Cu)	Pb	SD (Pb)	Cd	SD (Cd)
5 m	68.2	8.44	8.02	1.42	4.16	1.25	0.311	0.051
10 m	37.74	5.26	5.32	1.68	3.21	0.72	0.275	0.043
25 m	50.15	7.71	4.34	1.13	2.26	0.42	0.242	0.029
50 m	41.33	3.92	4.62	0.9	2.97	0.74	0.288	0.030
100 m	27.54	3.12	3.97	0.84	2.07	0.46	0.264	0.026

SD=standard deviation

As far as cadmium is concerned, the cadmium content of the plant samples did not change considerably between the distances of 5 and 100 metres, and there was no significant difference (Table 1). The values measured were higher than those of other authors (Table 2), indicating that, for the two most harmful heavy metals, while the concentration of lead has decreased (as observed in meadow plants along the motorway), the cadmium emission has remained unchanged.

An analysis of the SD values showed that in the case of plants located nearer the motorway the deviation from the average value was greater, indicating that the concentration changed over a wider interval. This could be related to the environmental pollution caused by vehicular traffic.

A comparison with the data of foreign authors is more difficult as their research was often carried out under completely different conditions (different climatic conditions, different traffic, different species and plant coverage). Olajire and Ayodele (1997) measured far higher element concentrations in plants found along roads than those measured in the present tests or by other Hungarian authors (Table 2).

Table 2

Heavy metal concentrations of plants found along roads (based on the data of several authors), mg kg^{-1} dry matter

		Zn	Cu	Pb	Cd
Data from Kádár (1995)	1 m	111	11	77	0.22
	5 m	31	5	22	0.10
	10 m	33	6	22	0.11
	30 m	30	6	16	0.11
	100 m	30	6	17	0.10
Data from Póti et al. (1997)	1 m	331	15	365	0.31
	5 m	120	13	32	0.17
	15 m	35	10	21	0.15
	50 m	35	11	20	0.16
	100 m	20	11	12	0.15
Average data from Olajire and Ayodele (1997)		59.6	44	291	0.77

Conclusions

Assessing the data of the experiment carried out in 2000, it can be concluded that the heavy metal concentrations of the plants in a field located 51 kilometres from Budapest along the M3 motorway decreased significantly for all the four metals under examination, with the exception of cadmium. The zinc and copper concentrations of the plant samples were similar to those published by other authors. In the case of lead, a considerable decline can be observed compared to the values published by other authors, presumably due to the wide use of lead-free fuels, introduced in 1996. By using fuels without lead additives the lead contamination of the environment decreases. Comparing these results with the national standards, it was found that the concentrations recorded never exceeded those set in the standards.

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Short communication

ASSOCIATION BETWEEN FIELD AND LABORATORY PREDICTORS OF DROUGHT TOLERANCE IN WHEAT DISOMIC ADDITION LINES

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In order to locate QTLs controlling field and laboratory indicators of drought tolerance, chromosome addition lines of *Agropyron elongatum* (donor) in the genetic background of Chinese Spring (recipient) were tested in the field and laboratory of the College of Agriculture, Razi University, Kermanshah, Iran. The plant genetic material was cultivated in the field and laboratory under two different water regimes (irrigated and non-irrigated).

High significant differences were found for promptness index (PI), coleoptile length (CL) and root length (RL) under stress and non-stress conditions, indicating the presence of genetic variation and the possibility of selection for these traits.

High correlation coefficients were found between PI, germination stress index (GSI) and stress tolerance index (STI), displaying a high association between the indices of field and laboratory predictors of drought tolerance. Field and laboratory predictors of drought tolerance showed that most of the QTLs controlling drought tolerance criteria in *Agropyron* are located on chromosomes 3E, 5E and 7E, which collectively constitute 84.3% of the additive genetic variance.

Key words: alien chromosome addition, drought tolerance, QTLs, stress tolerance index, germination stress index

Introduction

The evaluation of grain yield performance in areas exposed to frequent stress remains the most widely applied criterion for characterizing cultivar adaptation to stressful conditions. Breeding for drought tolerance by selecting solely for grain yield is difficult because the heritability of yield under drought conditions is low, due to small genotypic variance or to the large genotype-environment interaction variances (Blum, 1988; Ludlow and Muchow, 1990; Kőszegi et al., 1996).

In stressful environments, yield *per se* is not always the most suitable or easiest selection trait and an approach based on the evaluation of some of the physiological traits involved in stress tolerance was proposed (Blum, 1988). The incorporation of such attributes into a potentially high-yielding genotype may improve its adaptability and thus its response to environmental variability (Jaradat and Konzak, 1983; Steven et al., 1990). Screening techniques based on physiological criteria should be rapid, simple and inexpensive, especially for the evaluation of large populations (Gavuzzi et al., 1997). One of the screening

techniques based on physiological traits is the use of various osmotica to induce stress in plant tissues. Germination in mannitol and polyethylene glycol (PEG), measurements of root length or rooting depth, and the survival or growth of seedlings subjected to osmotica have been suggested for drought screening (Winter et al., 1988). Kaufman and Eckard (1971) and Leshem (1996) evaluated the effect of PEG on pepper, cucumber and *Pinus* and concluded that PEG was very suitable for the adjustment of osmotic potential.

The present investigation was carried out to locate QTLs controlling field and laboratory predictors of drought tolerance using disomic addition lines of *Agropyron elongatum* (Host.) Beauvois to *Triticum aestivum* L. em. Thell. cv. Chinese Spring.

Materials and methods

To locate QTLs controlling field and laboratory indicators of drought tolerance, disomic chromosome addition lines of *Agropyron elongatum* ($2n=2x=14$) into the genetic background of Chinese Spring (CS) wheat ($2n=6x=42$) and Sardari, an Iranian variety with good drought tolerance were used in different field and laboratory experiments. In the laboratory experiment 50 seeds from each disomic addition line were germinated in Petri dishes on wet filter paper. The Petri dishes were arranged in a completely randomized design with three replications under two different stress and non-stress water regimes in a growth chamber. Day and night temperatures were 15°C and 20°C, respectively, and the relative humidity was 75%. In the stress and non-stress treatments 10 ml of PEG with an osmotic potential of -8 Mpa and 10 ml of distilled water were added to the Petri dishes, respectively. After 10 days the number of germinated seeds was recorded and the promptness index (PI) and germination stress index (GSI) were calculated using the formulae suggested by Bouslama and Schapaugh (1984):

$$PI = nd_2 (1.0) + nd_4 (0.8) + nd_6 (0.6) + nd_8 (0.4) + nd_{10} (0.2)$$

where nd_2 , nd_4 , nd_6 , nd_8 and nd_{10} are the percentage of germinated seeds on the 2nd, 4th, 6th, 8th and 10th day, respectively.

$$GSI (\%) = \frac{(100)[(PI) \text{ under stress conditions}]}{[(PI) \text{ under non-stress conditions}]}$$

The characters coleoptile length, root length and root number were also measured.

Two different experiments were carried out in the field under irrigated and water stress conditions. The seeds were cultivated in a 1 m row with 3 × 25 cm plant and row distances. Using yield potential (Y_p) and stress yield (Y_s), the stress tolerance index (STI) was calculated with the formula suggested by Fernandez (1992):

$$STI = \frac{(Y_s) \times (Y_p)}{Y_p^2}$$

SPSS and MSTAT-C statistical softwares were used for the analysis of the data.

Results and discussion

The results of analysis of variance (Table 1) indicated highly significant differences for promptness index (PI), coleoptile length (CL), root length (RL) and root number (RN) under stress conditions, indicating the presence of genetic variation and the possibility of selection for laboratory predictors of drought tolerance.

Table 1

Analysis of variance for various characters investigated in the laboratory under stress conditions

Source of variation	D.F.	Mean squares			
		Promptness index	Coleoptile length	Root length	Root no.
Disomic additions	8	79.04**	1.14**	1.41**	0.163 ^{ns}
Error	18	1.61	0.03	0.052	0.10

** Significant at $p=0.01$; ns=non-significant

Mean comparison (Table 2) exhibited significant differences between the recipient (CS), 3E and 7E for PI, and between 3E, 5E and 7E for CL and RL, indicating that QTLs controlling PI, CL and RL are located on chromosomes 3E, 5E and 7E. Using the formula suggested by Kearsey and Pooni (1998):

$$a = \frac{Q^+Q^+ - Q^-Q^-}{2}$$

the additive effect of QTLs (a) on these chromosomes was 100% for PI, CL and RL. The importance of chromosomes 5E and 7E for drought tolerance was suggested by Sutka et al. (1995) and Farshadfar (1995). The importance of chromosomes 3E (Dvorak, 1993) and 5E (Mahmood and Quarrie, 1993) was also investigated for salt tolerance.

Multiple correlation analysis (Table 3) revealed a highly significant correlation between the laboratory (PI, GSI, CL and RL) and field (STI) predictors of drought tolerance, indicating that the germination stress index (GSI) can be screened as a drought tolerance criterion for the selection of drought-tolerant cultivars.

Mohammadi (1999) and Tikhonov (1973) found high correlation coefficients between PI, STI and GSI, which is in agreement with the results of this experiment. They also mentioned that the growth ability of the roots under stress conditions is an important factor in the survival and promptness index of the plant. Root length and coleoptile length were also screened as indices of drought tolerance (Bousslama and Schapaugh, 1984; Farshadfar et al., 1993; Farshadfar, 1995).

Table 2

Mean comparison between disomic addition lines and the recipient (Chinese Spring) for different characters under water stress conditions

Disomic additions	Promptness index	Coleoptile length	Root length	Root no.
1E	8.0de*	2.7d	3.4c	3.0a
2E	4.4f	2.3d	3.2cd	3.3a
3E	14.2b	3.4b	3.9b	3.0a
4E	5.7ef	2.3d	2.7d	3.0a
5E	13.0bc	2.9c	3.9b	3.3a
6E	6.9ef	2.5cd	3.0cd	3.0a
7E	13.8b	3.7ab	4.2b	3.6a
CS (Recipient)	12.2cd	2.4d	3.0cd	3.0a

*: Common letters mean no significant difference.

Table 3

Association between laboratory and field predictors of drought tolerance in disomic addition lines

Characters	GSI	PI	CL	RL	RN	STI
Germination stress index (GSI)	1	—	—	—	—	—
Promptness index (PI)	0.91**	1	—	—	—	—
Coleoptile length (CL)	0.86**	0.59	1	—	—	—
Root length (RL)	0.73*	0.42	0.94**	1	—	—
Root number (RN)	0.074	0.08	0.17	0.20	1	—
Stress tolerance index (STI)	0.85**	0.94**	0.59	0.39	0.30	1

*, **: Significant at $p=0.05$ and $p=0.01$, respectively

The values of PI, GSI and STI (Table 4) under stress conditions revealed that chromosomes 3E, 5E and 7E carry QTLs controlling these laboratory and field criteria of drought tolerance and can hence be used as the raw material for mapping and QTL analysis of drought tolerance using DNA markers and thereafter for marker-assisted selection. When using germination stress index (GSI) and stress tolerance index (STI) for cluster analysis on disomic addition lines by the UPGMA method, it was observed that chromosomes 3E, 5E and 7E were classified in one group, together with the donor *Agropyron elongatum* (Fig. 1).

Table 4

Values of promptness index, germination stress index and stress tolerance index for disomic addition lines and recipient (Chinese Spring)

Disomic additions	Promptness index	Germination stress index	Stress tolerance index
1 E	8.0	42.1	0.32
2 E	4.4	25.2	0.16
3 E	14.2	61.5	1.30
4 E	5.7	30.9	0.16
5 E	13.0	57.5	0.98
6 E	6.9	37.2	0.42
7 E	13.8	63.5	1.50
CS (Recipient)	10.2	44.2	0.77

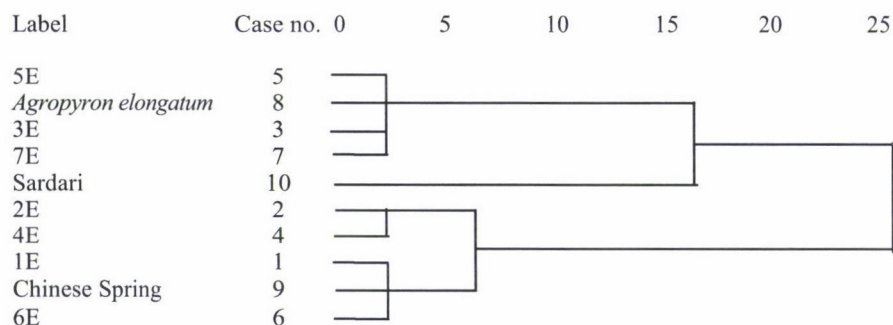


Fig. 1. Dendrogram resulting from cluster analysis on disomic additions based on germination stress index and stress tolerance index

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Short communication

PROTEIN AND WET GLUTEN CONTENTS IN WINTER
WHEAT GRAIN SAMPLES

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The milling and baking quality of wheat is mainly determined by the genetic basis, but may also be influenced by management techniques. Series of winter wheat varieties were examined under identical agronomic conditions in the experimental years of 1996–2001. Weed control, the rate and application time of nitrogen top dressing, the use of insecticide and fungicide and the climate of the production year were evaluated as main factors. In the present study two major characteristics: the protein and gluten content of grain samples, were examined.

The effect of nitrogen fertilization proved to have the strongest impact on both quality indexes. No significant quality differences were induced by the various means of weed control. Plant protection treatments had a rather diverse effect on the contents of the protein and gluten in certain years. The protein and gluten values were correlated in all the experimental treatments, but the level of the correlation showed considerable variation. The effect of crop year proved to be the strongest, followed by fertilization, from among the quality-determining factors. The protein versus gluten correlation was also found to be the closest in the case of nitrogen top dressing applications.

Key words: winter wheat, protein, gluten, agronomic impacts

Introduction

Winter wheat is one of the most important crops in Hungary with a high economic value (Ivány et al., 1994; Birkás, 1996). For this reason the improvement of wheat cultivation techniques is a major task in research and development. We are able to utilise a very wide range of wheat types because the various food and feed industries differ in their requirements. The use of wheat for flour milling has remained relatively stable in recent years (Bingham et al., 1985). The aim of wheat production is twofold: to provide quantity and quality (Bedő and Láng, 1997). The milling and baking quality of wheat is determined mainly by the genetic basis, but it can also be influenced by management techniques (Zhukovsky, 1954; Pollhamer, 1981; Ragasits, 1992; Vida et al., 1996). The aim of this study was to determine the role of some agronomic impacts on wheat quality. Since protein and gluten values, as main quality indicators, are manifested in fairly similar form, there is a need to gain more information concerning ways in which their behaviour differs (Kárpáti et al., 1996; Győri et al., 2002).

Materials and methods

A wide range of winter wheat varieties were examined under identical agronomic conditions in the experimental years of 1996–2001. Small-plot cereal management trials were run in the Nagygombos experimental nursery of the Crop Production Institute of Szent István University. The soil of the experimental field was a calcic chernozem (Calciustoll) with an average humus content of 2.4%. The experiments were conducted in a split-plot design with four replications and a plot size of 10 m². The plots were planted and harvested using plot machines. Various agronomic treatments were applied to the plots. Weed control, the rate and application time of nitrogen top dressing, the use of insecticide and fungicide and the climate of the production year were evaluated as main factors. Grain samples were taken from all the plots harvested. The present study evaluates the quality of the variety Alföld-90, a wheat produced as an agronomic standard in all experimental years. Milling and baking quality tests were done at the Concordia Laboratories and at the Crop Production Laboratory according to Hungarian standards (MSZ ISO 5531:1993, MSZ ISO 6645:1993).

Trial 1. Weed control effects

The herbicides were applied as provocative sprayings after heading (Feekes 9-10). All the pesticides were used at the recommended dose in order to study the effects of different herbicides on winter wheat. The trial had a double control; a weedy control with no protection and a hand-weeded control with no herbicide applications.

Herbicides applied and their active agents: Starane (fluroxipir) 0.8 l/ha, Pardner (bromoxynil) 2 l/ha, Banvel 480 (dicamba) 0.7 l/ha.

Trial 2. N top dressing effects

N fertilizer containing 34% ammonium nitrate was applied. The following doses were used in various splits:

1. Control (no chemical fertilizer)
2. 40 kg/ha N (t)
3. 40+40 kg/ha N (t+f)

Single top dressing treatments were carried out at tillering (t), while the split treatments were applied at tillering and flowering (t+f).

Trial 3. Plant protection effects

Fungicide and insecticide treatments were applied following the principles of integrated pest management. The treatments were as follows: untreated control, fungicide, insecticide, combined fungicide and insecticide.

Fungicides and active agents: Bayleton 125EC (triadimefon) 1/ha, Folicur TOP (tebuconazole+ triadimefon) 1/ha.

Insecticide and active agent: Bancol 50WP (bensultap) 0.75 kg/ha.

Results and discussion

Nitrogen fertilization proved to have the strongest effect on both quality parameters. No significant quality differences were induced by the type of weed control. Plant protection treatments had a rather diverse effect on protein and gluten contents in certain years. Tables 1–3 present the protein and wet gluten values recorded in the three trials.

Table 1
Protein and wet gluten contents (%) in winter wheat samples
(Trial 1. Weed control effects, Gödöllő–Nagyombos, 1996–2001)

Crop year	Weedy control		Hand-weeded control		Fluroxipir		Bromoxynil		Dicamba	
	Protein	Gluten	Protein	Gluten	Protein	Gluten	Protein	Gluten	Protein	Gluten
1996	15.5	40.2	15.8	40.3	14.9	37.4	15.8	38.5	15.8	40.6
1997	12.7	24.7	13.2	26.0	14.5	30.5	13.2	26.9	12.1	22.6
1998	12.9	28.6	13.1	27.8	12.5	27.5	11.5	24.4	12.4	27.6
1999	13.8	30.5	13.5	30.8	13.2	28.8	14.3	32.2	14.2	38.4
2000	11.8	29.0	11.5	27.7	12.3	30.1	11.6	28.3	10.8	25.7
2001	11.4	26.6	11.4	26.6	11.5	26.8	12.0	27.5	11.1	25.6
Mean	13.0	29.9	13.1	29.9	13.2	30.2	13.1	29.6	12.7	30.0

LSD_{0.95}: protein 1.09; gluten 4.42

Table 2
Protein and wet gluten contents (%) of winter wheat samples
(Trial 2. N top dressing effects; Gödöllő–Nagyombos, 1996–2001)

Crop year	Control		40 kg N		40+40 kg N	
	Protein	Gluten	Protein	Gluten	Protein	Gluten
1996	15.7	38.0	15.9	37.5	15.8	38.8
1997	13.0	25.4	13.6	26.7	14.1	29.5
1998	12.5	27.0	14.8	35.0	14.6	33.6
1999	15.5	32.8	16.5	35.0	18.8	39.8
2000	10.6	26.0	12.0	29.9	11.8	28.3
2001	11.7	28.4	10.9	25.7	17.4	39.2
Mean	13.2	29.6	14.0	31.6	15.4	34.7

LSD_{0.95}: protein 0.88; gluten 2.47

Table 3
Protein and wet gluten contents (%) of winter wheat samples
(Trial 3. Plant protection effects; Gödöllő–Nagyombos, 1996–2001)

Crop year	Control		Bensultap		Triadimefon + tebuconazole		Bensultap and triadimefon + tebuconazole	
	Protein	Gluten	Protein	Gluten	Protein	Gluten	Protein	Gluten
1996	15.4	38.3	15.5	39.9	15.7	40.5	15.8	38.5
1997	14.5	30.3	13.4	27.5	13.6	26.8	12.4	24.9
1998	11.0	24.4	12.7	28.5	12.2	27.5	12.3	26.7
1999	14.5	31.9	14.3	30.9	14.0	31.2	14.8	33.5
2000	11.9	28.4	11.1	28.1	12.2	30.2	11.1	28.1
2001	10.7	24.7	11.5	25.7	10.5	24.7	11.3	26.2
Mean	13.0	29.7	13.1	30.1	13.0	30.2	13.0	29.7

LSD_{0.95}: protein 1.10; gluten 2.93

The experimental years could be divided into two main groups: good and bad quality crop years. 1996 and 1999 were outstanding crop years for quality parameters, since both the protein and wet gluten values were good compared to the other years.

The protein and gluten values were correlated in all the experimental treatments, but the level of the correlations showed considerable variation (Table 4). Since the effect of crop year proved to be the strongest, followed by fertilization, from among the quality-determining factors, the protein versus gluten correlation was also found to be the closest in the case of nitrogen top dressing applications.

Conclusions

The use of different agrochemical treatments had various effects on wheat quality parameters. From among the treatments fertilization had the strongest effect on wheat quality, especially on protein content. Herbicides, fungicides and insecticides had occasional effects only. The results obtained suggest that agrochemical applications in general and N fertilization in particular may have an influence on quality. The protein and wet gluten contents of the wheat samples were closely correlated in most cases. However, in some experimental years and treatments this was not the case. Strong correlations were found mainly in "good quality" crop years.

Table 4
Correlations between protein and gluten contents
Gödöllő–Nagygyombos, 1996–2001

Treatment	r	P*
<i>Weed control effects</i>		
Weedy control	0.7142	+
Hand-weeded control	0.6857	+
Fluroxipir	0.8285	++
Bromoxynil	0.7714	++
Dicamba	0.7714	++
<i>N top dressing effects</i>		
Control	0.6000	-
40 kg nitrogen	0.8857	+++
40+40 kg nitrogen	0.9714	+++
<i>Plant protection effects</i>		
Control	0.9428	+++
Bensultap	0.7142	+
Triadimefon + tebuconazole	0.8571	++
Bensultap and triadimefon + tebuconazole	0.4857	-

*+, ++, +++: significant at P=10%; P=5%; P=1%, respectively

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NITRATE CONTENT IN LETTUCE (*LACTUCA SATIVA* L.) GROWN ON AEROPONICS WITH DIFFERENT QUANTITIES OF NITROGEN IN THE NUTRIENT SOLUTION

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The influence of different quantities of nitrogen in the nutrient solution on growth, development and nitrate content was studied in aeroponically grown lettuce (*Lactuca sativa* L.). Three successive experiments were conducted in 1999 from April to September, in an aeroponic system. The lettuce plants, cv. Vanity, were grown in aeroponics using four different amounts of nitrogen in the nutrient solutions. The pH level was maintained between 5.5 and 6.5, and the EC between 1.8 and 2.2 mS/cm. Fresh weight measurements were made on all the material. The differences between the mean fresh shoot weights were statistically significant in all three experiments. In the first experiment, the maximum final fresh weight in the treatment with 8 mM nitrogen averaged 999.0 g. In the second and third experiments the largest amount of nitrogen, 12 mM $\text{NO}_3\text{-N}$, significantly ($p < 0.05$) increased the fresh shoot weight of lettuce plants. Low levels of nitrate in the nutrient solution (4 mM $\text{NO}_3\text{-N}$ in the first and 1.2 mM $\text{NO}_3\text{-N}$ in the second aeroponic experiment) significantly ($p < 0.05$) increased the fresh weight of the final roots compared with the level of nitrate in standard nutrient solution (12 mM $\text{NO}_3\text{-N}$). The differences between the means for plant height were statistically significant ($p < 0.05$) in all three experiments. In the first and third experiments the lengths of the primary roots of the lettuce plants were significantly ($p < 0.05$) influenced by different $\text{NO}_3\text{-N}$ concentrations in the nutrient solution. The highest NO_3^- concentration in the lettuce leaves was recorded in plants grown in nutrient solutions with the highest $\text{NO}_3\text{-N}$ concentration (17 mM in the first, 12 mM in the second and third experiments). An acceptably low NO_3^- concentration was found in the leaves of lettuce treated containing with nutrient solution 4 mM $\text{NO}_3\text{-N}$ in all three experiments.

Key words: aeroponics, lettuce (*Lactuca sativa* L.), nitrogen, nitrate concentration

Introduction

The nitrate accumulation in plant tissues is an important aspect in vegetable growing. Several plant species accumulate NO_3^- as the result of absorbing more than can be reduced to ammonium. The accumulation of NO_3^- in plants depends on their morphological and genetic characteristics as well as on many environmental factors, such as nitrogen supply or methods of application, light intensity, photoperiod, temperature, water supply and competitive ions (Maynard et al., 1976). Growers can control only competitive ions, the amount of nitrogen and the source of nitrogen. Therefore there is a need to study methods by which the nitrate concentration can be lowered in leafy vegetables without affecting the yield (Gunes et al., 1994; Crawford, 1995; Giacomelli and Yuan, 1995; He and Lee, 1998).

Various strategies have been reported in the literature to decrease the concentration of nitrate in leafy vegetables. Immediate modifications in the N supply are only possible in hydroponics, so different hydroponic systems, such as NFT (Nutrient Film Technique), flowing culture units and aeroponics, have proven very useful as a tool for nutrient research. Soilless systems allow better control of plant mineral uptake through a suitable choice of nutrient solutions according to plant needs and growth stage (Fontes et al., 1997; Urrestarazu et al., 1998; Zhu et al., 2000). The methods used so far have mostly attempted to reduce the nitrate-nitrogen concentration in the nutrient solution a few days before harvesting (Santamaria et al., 1996a; 1997; Scaife, 1989). According to Andersen and Nielsen (1992), a decrease in the nitrate content in lettuce heads was achieved by growing plants in a soilless culture with a reduced supply of all nutrients. Similar results were obtained by Gunes et al. (1995) and Santamaria et al. (1996b) through the partial substitution of $\text{NO}_3\text{-N}$ by $\text{NH}_4\text{-N}$. However, these methods have not been able to match the requirements of high yield, high quality and, simultaneously, a low content of nitrate.

The subject of the research was to investigate the influence of different quantities of nitrogen in the nutrient solution on growth and nitrate accumulation in aeroponically grown lettuce (*Lactuca sativa* L.) cv. Vanity.

Materials and methods

Three successive experiments were conducted in an aeroponic system in a greenhouse located in the Experimental Field of the Biotechnical Faculty in Ljubljana (latitude: $46^\circ 04' \text{ N}$, longitude $14^\circ 31' \text{ W}$, above 300 m.a.s.l.), from April to September 1999.

The aeroponic system was made up of a structure of aluminium bars of rectangular section, $800 \text{ cm} \times 100 \text{ cm} \times 120 \text{ cm}$, covered with plastic film (inside black, outside white) to allow the recovery of the nutrient solution for recycling. A sixteen of expanded high density polystyrene panels, $100 \text{ cm} \times 50 \text{ cm} \times 3 \text{ cm}$, were placed on the metal construction. Two parallel rows of holes for the plants were made in each panel. Seeds of lettuce (*Lactuca sativa* L.) cultivar Vanity were sown in rockwool pots and watered daily with Resh's (Resh, 1995) nutrient solution at 0.5 mS cm^{-1} . Plants having three to four leaves were transferred to the aeroponic system. Each plot consisted of 2.0 m^2 of panel. The holes of the panel were far enough apart to give a density of 16 plants per square metre.

The roots of the plants were exposed continuously to the nutrient solutions that were circulated by means of a pump (Pedrollo pKm 65-1) and a plastic tube system, through which the solution was injected and leached to the bottom of a 120 l container and recirculated. An irrigation system was installed inside the structure, made up of two parallel pipes at 60 and 110 cm from ground level having microjet sprinklers with a flow rate of 35 l/h. The solution was sprayed for 1.5'–2.0', while stopping times were 5'–7'. The system was electronically controlled. The nutrient solution was prepared with tap water containing approximately (mg/l): 13.3 NO_3^- , 378.0 HCO_3^- , 0.25 PO_4^{3-} , 10.0 Cl^- , 27.0 SO_4^{2-} , 95.8 Ca^{2+} , 20.0 Mg^{2+} and 6.48 Na^+ .

Lettuce seedlings cv. Vanity were cultivated for 7 days in an aeroponic system using modified Resh's nutrient solution of the following composition (mmol/l): $5.5 \text{ Ca}(\text{NO}_3)_2$, $1.6 \text{ KH}_2\text{PO}_4$, $1.9 \text{ K}_2\text{SO}_4$, $1.8 \text{ NH}_4\text{NO}_3$, $1.6 \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, $9.0 \times 10^{-2} \text{ Fe-Sequestrene}$, $1.6 \times 10^{-3} \text{ CuSO}_4 \cdot 5\text{H}_2\text{O}$, $0.5 \times 10^{-3} \text{ MoO}_3$, $9.1 \times 10^{-3} \text{ MnSO}_4$, $1.5 \times 10^{-4} \text{ ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and $1.4 \times 10^{-2} \text{ H}_3\text{BO}_4$.

Thereafter the $\text{NO}_3\text{-N}$ amounts were changed to the following treatments:

First experiment: 12.0 mmol/l (A), 17.0 mmol/l (B), 8.0 mmol/l (C), 4.0 mmol/l (D)

Second experiment: 1.2 mmol/l (A), 0.5 mmol/l (B), 4.0 mmol/l (C), 12.0 mmol/l (D)

Third experiment: 12.0 mmol/l (A), 8 mmol/l (B), 4 mmol/l (C), 1.2 mmol/l (D)

Each experiment involved 32 lettuce plants. The plants were grown under natural daylight conditions. During the trial, the quality of the nutrient solution was checked daily; its concentration was measured with a conductometer and kept between 1.8 and 2.2 mScm⁻¹. The acidity of the nutrient solution was also measured daily and balanced in the range of pH 5.5 and 6.5 by the addition of sulphurous acid. The nutrient solution was changed twice a week.

The experiments comprised four replicates, the produce of which was harvested 30 and 33 days after the transfer of the plants to the aeroponic system. The shoots and roots were separated and individual plants were weighed and dried for 4 days at 50°C. The treatment averages were analysed using the Duncan test. The dried matter was ground and analysed for nitrate, ammonium and total nitrogen concentration in an aqueous extract using an Autoanalyser II (Braun and Luebbe) and Kjeltec.

Results and discussion

Nitrate accumulation in crop plants is undesirable for a number of reasons. Nitrate, once ingested, may be converted to nitrite and result in the formation of compounds (methaemoglobinemia and nitrosamines) harmful to human health (Reinink and Groenwold, 1988), so various strategies have been developed to decrease the concentration of nitrate in leafy vegetables. According to Santamaria et al. (1996a) and Andersen and Nielsen (1992) the nitrate concentration can be reduced by eliminating NO₃-N from the nutrient solution or replacing it with chloride (Cl⁻), sulphate (SO₄⁻) or NH₄⁺ a few days before crop harvesting. However, these strategies have not been able to match the requirements of a high quantity and quality of lettuce yield.

In the present experiments an attempt was made to investigate the influence of different nitrogen amounts in the nutrient solution on the growth and nitrate accumulation in aeroponically grown lettuce (*Lactuca sativa* L.) cv. Vanity.

The effects of different NO₃-N concentrations in the nutrient solution on the shoot and root fresh weight are illustrated for each individual experiment in Figures 1, 2 and 3. Significant differences between the treatments were calculated using Duncan's multiple range test ($p < 0.05$).

An increase in the NO₃-N availability in the nutrient solution affected the growth and development of lettuce plants grown in an aeroponic system. In the first experiment, which lasted from April to June 1999, the differences between the mean values of shoot fresh weight were statistically significant ($p < 0.05$) (Fig. 1). The maximum final fresh shoot weight, obtained in the 8 mM treatment, was 12% higher on average than the second greatest fresh shoot weight, found in the 12 mM treatment, 25% higher than that in the 17 mM treatment and 57% higher than that in the 4 mM treatment. In the second and third experiments, carried out in June to July and July to August 1999 (Figs. 2 and 3), the largest amount of nitrogen in the nutrient solution (12 mM NO₃-N in the second and 12 mM and 8 mM NO₃-N in the third experiment) significantly ($p < 0.05$) increased the fresh shoot weight of the lettuce plants.

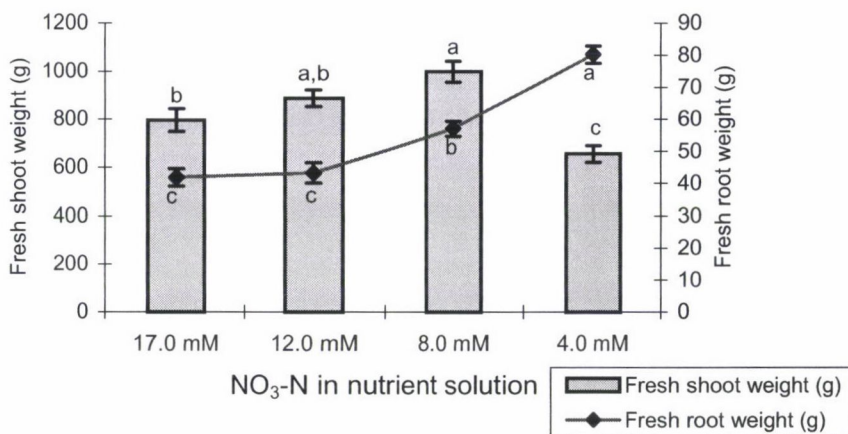


Fig. 1. Fresh shoot and root weights of lettuce grown in an aeroponic system with different NO₃-N concentrations in the nutrient solution in an experiment lasting from April to June 1999. Vertical bars represent the standard error.

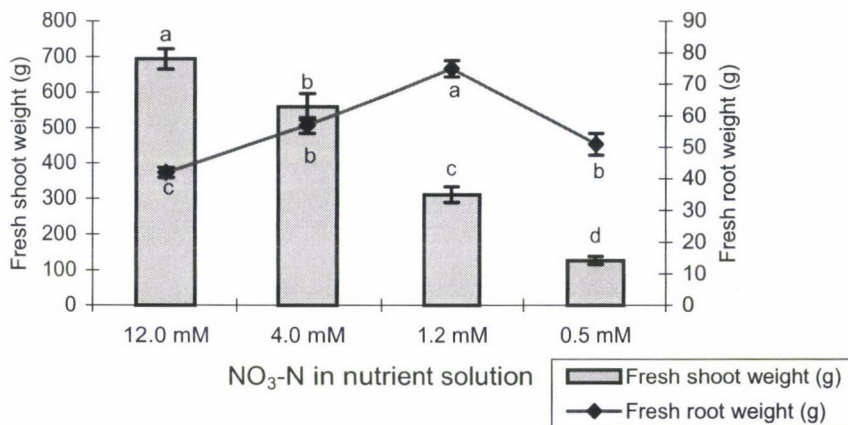


Fig. 2. Fresh shoot and root weights of lettuce grown in an aeroponic system with different NO₃-N concentrations in the nutrient solution in an experiment lasting from June to July 1999. Vertical bars represent the standard error.

In the first two experiments a low level of NO₃-N in the nutrient solution (4 mM NO₃-N in the first and 1.2 mM in the second) significantly ($p < 0.05$) increased the fresh weight of the final roots compared to the level of NO₃-N in standard nutrient solution (12 mM NO₃-N). A similar effect of a decrease in the nitrate concentration in the nutrient solution on the biomass partitioning between the shoots and roots was found by Ameziane et al. (1995; 1997) and Zerihun et al. (1998).

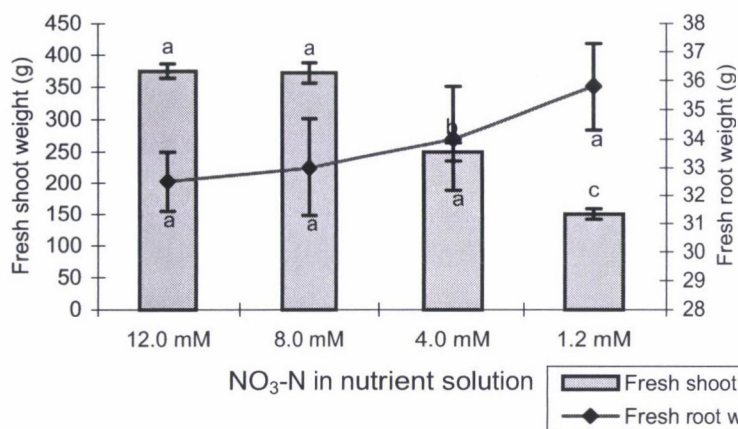


Fig. 3. Fresh shoot and root weights of lettuce grown in an aeroponic system with different NO₃-N concentrations in the nutrient solution in an experiment lasting from July to August 1999. Vertical bars represent the standard error.

In agreement with findings that nitrogen deficiency shifts the shoot/root ratio in favour of root growth (Rufty et al., 1984), it was found that under conditions of N -deficiency (4 mM NO₃-N in the first experiment, 4 mM and 1.2 mM NO₃-N in the second, 1.2 mM NO₃-N in nutrient solution in the third), which increased the root growth, the shoot growth was dramatically diminished. Thus, a decrease in the nitrogen supply resulted in a marked decrease in the shoot/root ratio in all three experiments.

The values of the plant height, rosette diameter and the length of the primary root are given for each individual experiment in Tables 1, 2 and 3. Significant differences between the treatments were calculated according to Duncan's multiple range test ($p < 0.05$).

In all three experiments the differences between the mean values of plant height were statistically significant ($p < 0.05$). The lettuce heads were less compact when the plants were grown in nutrient solution a with high concentration of NO₃-N (17 mM and 12 mM in the first, 12 mM and 4 mM in the second, and 12 mM and 8 mM NO₃-N in the nutrient solution in the third experiment) than in plants grown at a low concentration of NO₃-N.

In the first experiment (Table 1) the smallest plants with the longest root system were produced by the lowest (4 mM) NO₃-N concentration in the nutrient solution. Similar trends in the plant height and the length of the primary root were observed in lettuce plants grown in the other two experiments (Tables 2 and 3), where the NO₃-N concentration in the nutrient solution varied from 0.5 to 12 mM.

An important parameter in lettuce quality is the concentration of nitrogen compounds in the fresh yield of lettuce. The nitrate, ammonium and total nitrogen content was measured in the leaves of lettuce plants. The effects of different NO₃-N concentrations in the nutrient solution on the nitrate, ammonium (expressed in terms of fresh matter) and the total nitrogen content are presented for each individual experiment in Tables 4, 5 and 6. The differences were tested with the LSD test at the 0.05% level of probability.

Table 1

Plant height (cm), rosette diameter (cm) and length of primary root (cm) of lettuce plants cv. Vanity grown on aeroponics with different $\text{NO}_3\text{-N}$ concentrations in the nutrient solution in an experiment conducted from April to June 1999

$\text{NO}_3\text{-N}$ concentration in the nutrient solution	Average plant height	Average rosette diameter	Average length of primary root
17 mM	27.0a*	22.3bc	74.1b
12 mM	26.8a	24.4a	72.2b
8 mM	24.2b	23.4ab	69.1b
4 mM	20.8c	21.7c	90.0a

*Means in a column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's multiple range test ($p < 0.05$), $n=4$

Table 2

Plant height (cm), rosette diameter (cm) and length of primary root (cm) of lettuce plants cv. Vanity grown on aeroponics with different $\text{NO}_3\text{-N}$ concentrations in the nutrient solution in an experiment conducted from June to July 1999

$\text{NO}_3\text{-N}$ concentration in the nutrient solution	Average plant height	Average rosette diameter	Average length of primary root
12.0 mM	23.0a*	22.6a	92.0b
4.0 mM	22.0a	24.1a	110.0a
1.2 mM	17.0b	23.0a	106.0a
0.5 mM	12.0c	17.5b	95.0b

*Means in a column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's multiple range test ($p < 0.05$), $n=4$

Table 3

Plant height (cm), rosette diameter (cm) and length of primary root (cm) of lettuce plants cv. Vanity grown on aeroponics with different $\text{NO}_3\text{-N}$ concentrations in the nutrient solution in an experiment conducted from July to August 1999

$\text{NO}_3\text{-N}$ concentration in the nutrient solution	Average plant height	Average rosette diameter	Average length of primary root
12.0 mM	25.0a*	26.0a	82.0b
8.0 mM	25.0a	26.0a	83.0b
4.0 mM	22.0b	23.0b	87.0b
1.2 mM	15.0c	20.0c	96.0a

*Means in a column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's multiple range test ($p < 0.05$), $n=4$

Table 4

Contents of nitrate (NO_3^-), ammonium (NH_4^+) and total nitrogen (N) in fresh leaves of aeroponically grown lettuce in an experiment lasting from April to June 1999

$\text{NO}_3\text{-N}$ in the nutrient solution	Average content of nitrogen compounds in lettuce leaves		
	NO_3^- (mg/kg FM)	NH_4^+ (mg/kg FM)	N (g/kg)
17 mM	1618.3c*	37.5b	32.7a
12 mM	1681.8b	42.2a	34.0a
8 mM	1966.2a	33.7c	33.0a
4 mM	689.0d	12.5d	20.0b

* Means in a column followed by the same letter are not significantly different at the 5% level of probability (LSD test, $p < 0.05$), $n=4$

Table 5

Contents of nitrate (NO_3^-), ammonium (NH_4^+) and total nitrogen (N) in fresh leaves of aeroponically grown lettuce in an experiment lasting from June to July 1999

$\text{NO}_3\text{-N}$ in the nutrient solution	Average content of nitrogen compounds in lettuce leaves		
	NO_3^- (mg/kg FM)	NH_4^+ (mg/kg FM)	N (g/kg)
12.0 mM	1860.3a*	42.8a	32.9a
4.0 mM	650.7b	29.2b	26.5b
1.2 mM	37.8c	12.3c	16.3c
0.5 mM	5.4d	7.7d	14.0d

* Means in a column followed by the same letter are not significantly different at the 5% level of probability (LSD test, $p < 0.05$), $n=4$

Table 6

Contents of nitrate (NO_3^-), ammonium (NH_4^+) and total nitrogen (N) in fresh leaves of aeroponically grown lettuce in an experiment lasting from July to August 1999

$\text{NO}_3\text{-N}$ in the nutrient solution	Average content of nitrogen compounds in lettuce leaves		
	NO_3^- (mg/kg FM)	NH_4^+ (mg/kg FM)	N (g/kg)
12.0 mM	2552.7a*	35.0c	42.5a
8.0 mM	2357.2b	55.7a	42.8a
4.0 mM	1082.5c	39.5b	35.9b
1.2 mM	203.7d	14.5d	23.2c

* Means in a column followed by the same letter are not significantly different at the 5% level of probability (LSD test, $p < 0.05$), $n=4$

In all three aeroponic experiments the highest nitrate content was recorded in the leaves of lettuce plants grown in the nutrient solution with the highest $\text{NO}_3\text{-N}$ concentration (17 mM in the first, 12 mM in the second and third experiments), whereas the lowest content was recorded in the leaves of lettuce plants grown on aeroponics with a low $\text{NO}_3\text{-N}$ concentration. These results are in agreement with those of other authors (Steingröver et al., 1986; Marschner, 1996) who suggested that in treatments with low $\text{NO}_3\text{-N}$ in the nutrient solution a great part of the $\text{NO}_3\text{-N}$ was assimilated in the metabolic pool, whereas little remained available for accumulation in the reserve pool or in cell vacuoles as osmotic regulator.

The results in Table 4 show that the nitrate content was greatest (1996.2 mg/kg FM) when lettuce plants were grown on aeroponics with 8 mM $\text{NO}_3\text{-N}$ in the nutrient solution in the experiment conducted from April to June 1999.

The nitrate content was extremely high in the leaves of lettuce plants from the third experiment (Table 6) when the $\text{NO}_3\text{-N}$ concentration in the nutrient solution was 12 mM or 8 mM but was more than halved when the available $\text{NO}_3\text{-N}$ in the nutrient solution was 4 mM. It is thought that the high nitrate levels in the leaves were associated with a period of extremely high air temperature (33–35°C) and nutrient solution temperature (29–32°C), so that the nitrate assimilation rate was restricted.

In the second experiment, conducted from June to July 1999, lettuce plants grown on aeroponics with the lowest nitrogen level in the nutrient solution (0.5 mM $\text{NO}_3\text{-N}$) had a higher ammonium concentration in the leaves than the nitrate concentration. It seems that the amount of $\text{NO}_3\text{-N}$ in the nutrient solution was too small to meet the demands for nitrate reduction and assimilation into the organic compound.

Conclusions

On the basis of the experiments it can be concluded that the nitrogen concentration in the nutrient solution had a great influence on the growth and development of lettuce plants grown in an aeroponic system.

In the experiment lasting from April to June, the elongation of the lettuce stems was promoted and the formation of compact heads was absent in plants whose roots were exposed to higher $\text{NO}_3\text{-N}$ concentrations (17 mM and 12 mM). On the other hand, the lettuce heads were more compact when the plants were grown in nutrient solution with 8 mM $\text{NO}_3\text{-N}$. According to these results, when lettuce is cultivated in an aeroponic system, a $\text{NO}_3\text{-N}$ concentration of 8 mM in the nutrient solution is optimum for the early summer growing period.

The experiments also attempted to assess whether the NO_3^- content could be reduced in aeroponically grown lettuce by decreasing the $\text{NO}_3\text{-N}$ in the nutrient solution. The statistical evaluation of the NO_3^- content in lettuce plants showed that a sufficiently low NO_3^- concentration was found in the leaves of lettuce plants grown on nutrient solution with 4 mM $\text{NO}_3\text{-N}$ in all three experiments.

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PHOTOSYNTHETIC PRODUCTIVITY OF SIX WINTER WHEAT GENOTYPES (*Triticum aestivum* L.)

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Differences between six new winter wheat genotypes for some photosynthetic parameters, namely leaf area, leaf area index (LAI), specific leaf area (SLA), dynamics of chloroplast pigment concentration and of dry matter formation, were investigated at two localities, Donji Miholjac and Kutjevo, during the vegetation periods 1997/98 and 1998/99. The parameters tested were determined by standard methods. The greatest differences between the photosynthetic productivity parameters of the genotypes investigated were determined from the heading stage to the milky ripe stage. The relationship between the dry matter accumulation and other physiological parameters, e.g. chloroplast pigment concentration, LAI and SLA, is stable and significant, but strongly influenced by external factors. Statistical analyses showed a very significant relationship between the dry matter content and the parameters examined, with a large number of significant correlations. The most important positive correlation was found between the dry matter content and the content of chlorophyll *a*, *b* and carotenoids, while negative correlations were found with LAI and SLA. The photosynthetic parameters investigated were also negatively correlated with SLA.

Key words: *Triticum aestivum* L., genotype, chloroplast pigments, leaf area index, specific leaf area, dry matter

Introduction

The chloroplast pigment content exhibited an increase in all phenophases in highly productive wheat varieties having higher photosynthesis intensity, and the *Triticum aestivum* species had a higher content of chloroplast pigments compared to the *Triticum boeoticum* D (Kovtun, 1974). Differences in this influence are especially pronounced for physiological processes affected by the genotype and by nitrogen nutrition (Pavlov, 1984). In order to achieve high wheat yields it is important to ensure the photosynthetic rate by maintaining a high leaf area index (LAI) till late maturity (Osaki et al., 1991). Optimal LAI (leaf area index) differs with the genotype and the agroecological conditions, and both too high and too low LAI values bring about yield reduction (Peltonen-Sainio et al., 1997). An increase in nitrogen top dressing in spring brings about an increase in LAI, resulting in a slowing down of leaf area loss, in turn having a slight impact on grain filling duration (Frederick, 1997). The photosynthesis intensity in wheat ranges between 20–40 mg CO₂/dm²/ha depending on the leaf mesophyll rate. Thus, it was determined that genotypes having a lower specific leaf area value (SLA) had higher photosynthesis (Dubravec and Regula, 1995). Osaki et al. (1991) found no correlation between SLA and the biological and

economic yield of the cultivars investigated. They suggested that the yield of the cultivars was not limited by the plant structure but affected by physiological plant processes. The period characterized by the most pronounced wheat variety specificity in terms of dry matter accumulation is the intensive growth period, i.e. from the beginning of tillering to the flowering stage (Waldern and Flowerdy, 1979). According to Cox et al. (1985) data on dry matter accumulation in various wheat growth stages are required for an understanding of the assimilation process as well as the share of carbon and nitrogen in plant growth. This paper aims to determine if there are any differences in morphological and physiological traits and productivity between six new winter wheat genotypes, based on differences in photosynthetic parameters.

Materials and methods

The research included six new genotypes of winter wheat: Lara, Lenta, Kruna, Fiesta, AG-45 and Perla, grown at two locations, near Donji Miholjac and Kutjevo (in Slavonia). The research was done in two vegetation periods, 1997/98 and 1998/99. The characteristics and genetic background of the wheat genotypes varied. The wheat genotypes examined differed especially in the grain yield (Table 7). The varieties Žitarka, Slavonia, Njivka, Pitoma, Gemini, OSK. 7-5/3-82, X-87-83x3.68/2-81 and GO-3135 were used to create the genotypes examined. The chosen locations differed in the height above sea level, the chemical content of the soil, the fertilizer use and the climatic conditions. Donji Miholjac is located 86 m.a.s.l. in a distinctly low-lying area, whereas Kutjevo is on undulating ground located 236 m.a.s.l. Differences in the chemical composition of the soil and the fertilizers applied are shown in Tables 1 and 2.

Table 1
Results of soil analysis at the locations investigated

Locations	pH H ₂ O	pH HCl	Humus (%)	P ₂ O ₅	K ₂ O
				mg kg ⁻¹	
Donji Miholjac	5.70	4.43	1.47	146.7	180.8
Kutjevo	7.10	6.03	1.47	305.6	158.2

Table 2
Fertilization (kg/ha) applied at the locations in 1997/98 and 1998/99

Locations	N			P ₂ O ₅	K ₂ O
	Basic dressing	Top dressing	Σ	Σ	Σ
1997/98					
Donji Miholjac	107	82	189	67.5	52.5
Kutjevo	40	117	157	120	80.0
1998/99					
Donji Miholjac	126	68.5	194.5	67.5	52.5
Kutjevo	121	95.0	176.0	120	80.0

The average monthly temperatures in Donji Miholjac ranged from -0.3°C (December 1997) and -1.1°C (December 1998) to 20.8°C (July 1998) and 22.4°C (July 1999), while in Kutjevo the temperatures ranged from -0.6°C (December 1997) and -1.1°C (December 1998) to 20.3°C (July 1998) and 22.3°C (July 1999). The differences in the amount of precipitation were greater than the temperature differences. The mean monthly precipitation in Donji Miholjac was 66.7 mm/m^2 in December 1997 and 92.4 mm/m^2 in December 1998. These values were much higher in Kutjevo and ranged from 126.0 mm/m^2 and 114.5 mm/m^2 (December 1997 and 1998) to 147.3 mm/m^2 and 127.6 mm/m^2 in July 1998 and 1999. The average air humidity in Donji Miholjac was 91% and 86% in December 1997 and 1998, while in July 1998 and 1999 it was 67% and 66%. The humidity in Kutjevo was 94% and 93% in December 1997 and 1998 and 72% and 74% in July 1998 and 1999, respectively.

Plant samples were taken seven times during the wheat vegetation season in 1997/98 and 1998/99, i.e. in the tillering stage (stages 2 and 5), the heading stage (stages 6 and 10), the earing stage (stage 10.1), the flowering stage (stage 10.5) and the milky ripe stage (stage 11). The wheat stages were determined on the Feekes scale. The following wheat qualities were examined: leaf area, leaf area index, specific leaf area, chloroplast pigment concentration and dry matter content of the aboveground wheat organs.

1. The leaf area was determined by the method of leaf contours on the paper, based on the leaf area of five average plants ($\times 4$) and the total number of plants per unit area. Twenty samples were taken from each genotype and the leaf area was given in m^2/ha (Sarić et al., 1986).

2. The leaf area index (LAI) was computed from the total leaf area and the soil area and was expressed in m^2/m^2 soil.

3. The specific leaf area was determined on the basis of the total leaf area of the plants and the leaf dry matter. Twenty samples ($\times 4$) were taken from each genotype and expressed in m^2/kg dry matter.

4. The chlorophyll pigments were determined on 0.1 g samples of fresh material from the most developed leaf on the primary wheat stalk. The concentrations of chl *a*, chl *b* and carotenoids were determined spectrophotometrically (at wavelengths of 662, 644 and 440 nm) from an acetone extract using the methods of Holm and Wettstein and expressed in mg/g of dry mass (Sarić et al., 1986)

5. The dry matter of the aboveground plant organs (kg/ha) was determined in terms of the dry matter mass of 20 average plants ($\times 4$) and of the total number of plants per unit area taken during the earing stage. The total number of plant samples taken from each wheat genotype was 80. The plant material was dried at 105°C until constant weight was achieved.

6. The grain yield was determined on the basis of the grain weight of 20 ears ($\times 4$) and the number of plants per unit area determined in the earing stage (stage 10.1) and was expressed in tonnes per hectare. The total number of plant samples taken from each genotype was 80.

7. Biological yield was determined on the basis of the weight of 20 plants ($\times 4$) and the number of plants per unit soil area determined in the earing stage (stage 10.1) and was expressed in tonnes per hectare. Eighty plants were sampled from each genotype.

8. The influence of genetic specificity and the agroecological conditions of the locality during the phenophases on the photosynthetic productivity parameters of wheat was investigated by variance analysis and tested with the F-test. The significance of the differences between genotypes, phenophases and locations was determined by the LSD test ($P_{0.05^*}$; 0.01^{**}). Mutual interdependence between the parameters was demonstrated by multiple regression and correlation analysis.

Results

In 1997/98 the leaf area of the genotypes examined increased at both locations from the tillering stage (stage 2) to the shooting stage (stage 10) (Fig. 1), while in 1998/99 the area constantly increased from the tillering stage (stage 2) to the earing stage (stage 10.1) (Fig. 2).

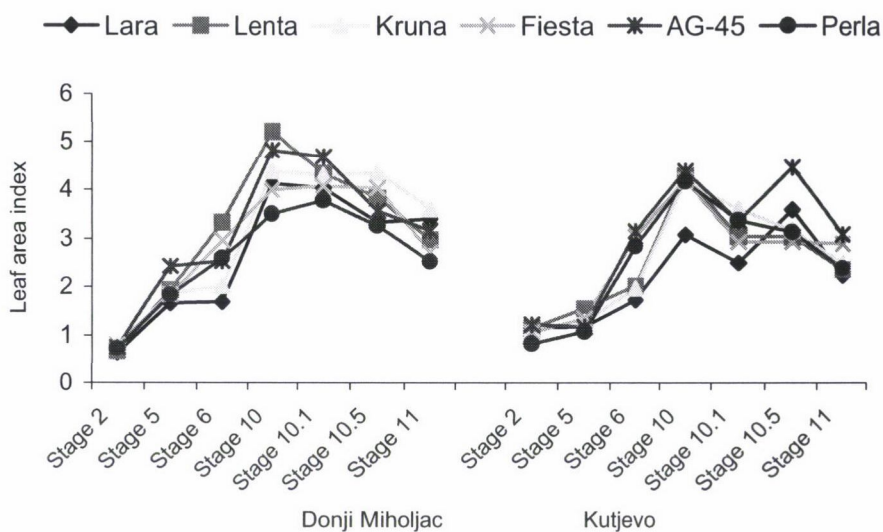


Fig.1. Average values of leaf area index (LAI) (m^2/m^2) of the investigated genotypes per phenophase (stage) at both locations in 1997/98

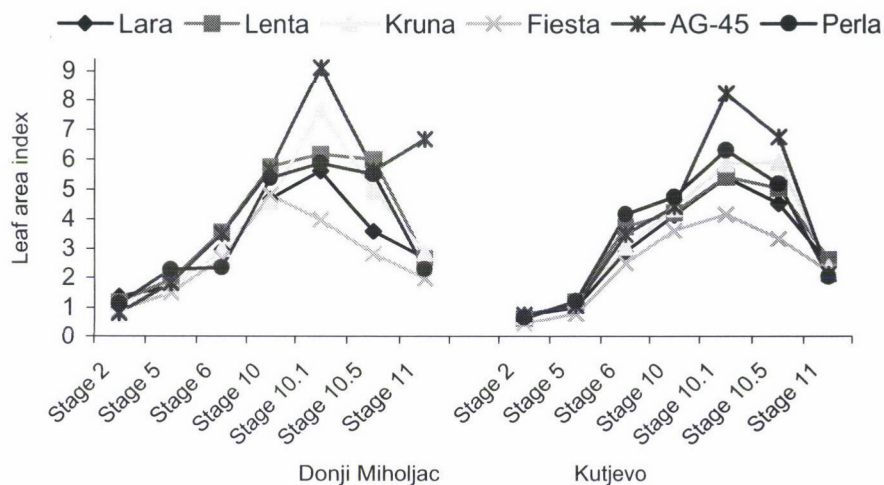


Fig. 2. Average values of leaf area index (LAI) (m^2/m^2) of the investigated genotypes per phenophase (stage) at both locations in 1998/99

The increase in the leaf area was greater in Donji Miholjac in both years due to the more favourable N fertilization (larger amount of N during basic fertilization and additional plant nutrition, Table 2). In 1997/98 the leaf area decreased from the shooting stage (stage 10) until the milky ripe stage (stage 11), depending on the genotype and location (Fig. 1), while in 1998/99 the decrease started during the earing stage (stage 10.1) and lasted until the milky ripe stage (Fig. 2). The very important influence of location, phenophase and genotype, as well as the significant interaction between the location, phenophase and genotype on the leaf area development was determined by variance analysis in both experimental years (Tables 3 and 4).

Significant differences in leaf area development, i.e. leaf area index, between the genotypes and locations investigated were determined in the milky ripe stage (stage 11) in both experimental years, with a variability coefficient of 60.33% (Figs. 1 and 2).

Table 3

Variance analysis for photosynthetic parameters involved in wheat productivity (*F*-test) in the genotypes examined at both locations in 1997/98 ($P < 0.05^*$; $P < 0.01^{**}$)

Parameters	Location (L)	Genotype (G)	Phenophase (P)	L×G	L×P	G×P	L×G×P
Chl. <i>a</i>	258.607**	13.854 ^{NS}	313.576**	3.878**	23.158**	2.630**	4.242**
Chl. <i>b</i>	21.527*	3.720**	146.132**	3.176*	43.128**	1.859**	3.579**
Carotenoids	106.272**	18.356 ^{NS}	216.998**	4.197**	31.338**	2.410**	4.173**
Leaf area	566.931**	8.894**	430.709**	4.177**	17.683**	2.952**	3.475**
LAI	93.864**	12.589**	421.569**	4.545**	16.125**	3.256**	3.446**
SLA	91.693**	10.927**	204.617**	5.103**	17.027**	3.586**	2.778**
Dry matter	425.263**	33.826**	1456.573**	9.852**	48.910**	3.105**	2.390**

*Significant at the 5% level; **significant at the 1% level, ^{NS} not significant

Table 4

Variance analysis for photosynthetic parameters involved in wheat productivity (*F*-test) in the genotypes examined at both locations in 1998/99 ($P < 0.05^*$; $P < 0.01^{**}$)

Parameters	Location (L)	Genotype (G)	Phenophase (P)	L×G	L×P	G×P	L×G×P
Chl. <i>a</i>	21.692**	26.387 ^{NS}	119.565**	2.001 ^{NS}	12.330**	5.885**	9.277**
Chl. <i>b</i>	357.561**	6.933**	55.453**	2.758**	5.069**	2.747**	5.546**
Carotenoids	4.689**	4.797 ^{NS}	73.611**	2.123 ^{NS}	14.382**	4.323**	8.364**
Leaf area	110.516**	29.770**	730.387**	3.005*	12.593**	10.644**	4.030**
LAI	79.631**	118.609**	882.891**	4.891**	12.191**	15.407**	3.585**
SLA	39.047**	5.265**	133.623**	3.612 ^{NS}	3.658**	1.777**	2.547**
Dry matter	52.564**	22.856**	3695.585**	4.189**	35.661**	3.927**	2.731**

*Significant at the 5% level; **significant at the 1% level, ^{NS} not significant

The highest SLA values were recorded for the genotypes in the tillering stage (stage 2) when the highest differences were determined between the investigated genotypes and locations at both locations and in both experimental years, with a variability coefficient of 19.9% (Fig. 3). The very important influence of the phenophase, location and genotype on the SLA was determined by the analysis of variance and the interactions of locations, phenophases and genotypes were also important (Tables 3 and 4). The strong interaction determined between the location and the phenophase on SLA could be an indication of the different mineral nutrient supplies and climatic conditions of the wheat growing location, while the strong interaction determined between genotype and phenophase on SLA could be explained by the different genotypic backgrounds of the varieties (Tables 3 and 4).

The photosynthetic pigment contents of the genotypes were highest during the flowering stage (stage 10.5) in both experimental years at both locations. Significant differences in chloroplast pigment concentration were determined between the genotypes and locations in the milky ripe stage (stage 11), with a variability coefficient of 46.20% (Figs. 4, 5 and 6). A very significant influence of the phenophase and the locations on the chlorophyll *a* content was determined by the *F*-test in both years, whereas the effect of genotype was not statistically significant (Tables 3 and 4). In 1997/98 the dynamics of chlorophyll *b* content depended on the phenophase and the genotype, while the effect of the location was not significant (Table 3). Location, genotype and phenophase were shown by variance analysis to have a significant influence on the chlorophyll *b* content in the second experimental year (Table 4). A very significant influence of the phenophase and the locations on the carotenoid concentration was determined by the *F*-test in both years, while the genotype effect was not statistically significant (Tables 3 and 4).

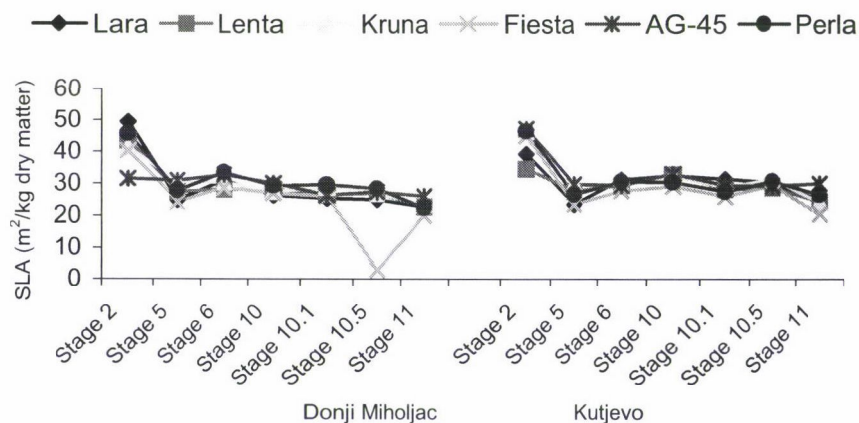


Fig. 3. Average values of specific leaf area (SLA) (m²/kg dry matter) of the investigated wheat genotypes per phenophase (stage) at both locations in 1997/98 and 1998/99

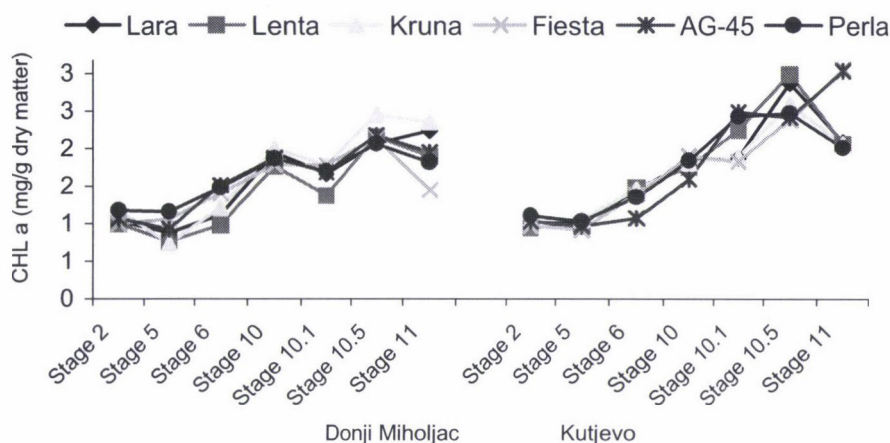


Fig. 4. Average values of leaf chlorophyll *a* concentrations (mg/g dry matter) of the investigated wheat genotypes per phenophase (stage) at both locations in 1997/98 and 1998/99

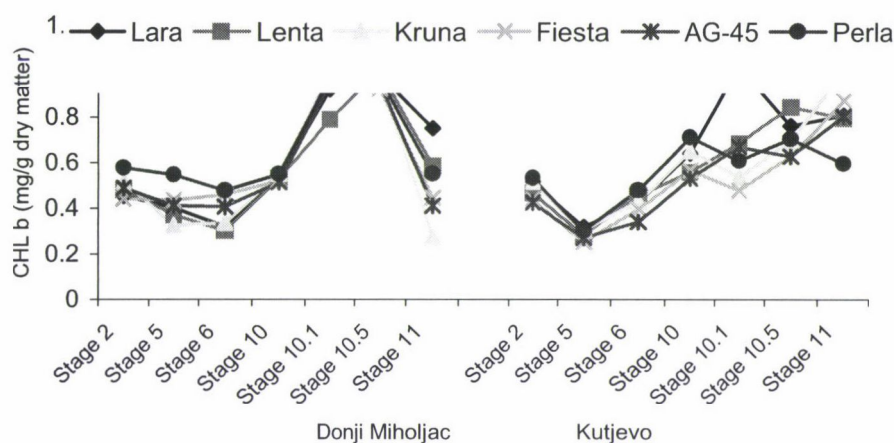


Figure 5. Average values of leaf chlorophyll *b* concentrations (mg/g dry matter) of the investigated wheat genotypes per phenophase (stage) at both locations in 1997/98 and 1998/99

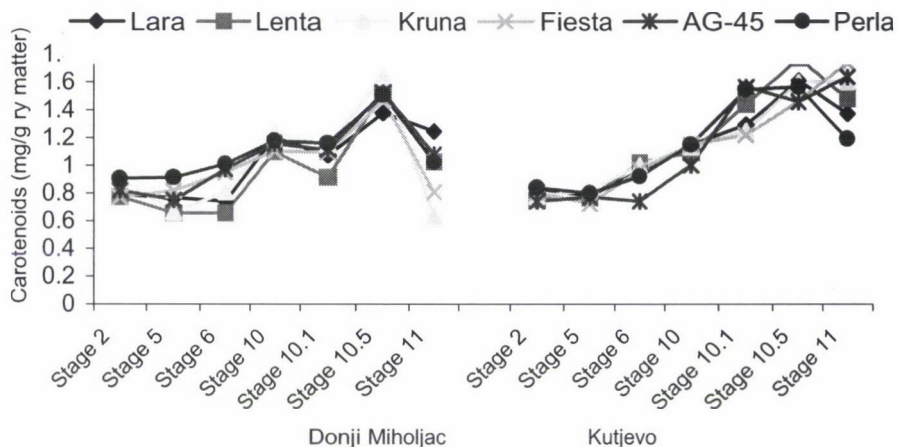


Figure 6. Average values of leaf carotenoid concentrations (mg/g dry matter) of the investigated wheat genotypes per phenophase (stage) at both locations in 1997/98 and 1998/99

The dynamics of dry matter increase was similar to that of leaf area increase at both locations in both experimental years. As wheat matured the dry matter of the aboveground parts constantly increased (Fig. 7). The greatest differences in dry matter accumulation between the genotypes and locations were recorded in the milky ripe stage (stage 11), with a variability coefficient of 71.34% (Fig. 7) in both experimental years. Phenophases, locations and genotype were found by variance analysis to have a highly significant influence on dry matter accumulation in the wheat genotypes investigated in 1997/98 and 1998/99. The interactions between location, phenophase and genotype were also very significant (Tables 3 and 4).

Discussion

The leaf area was considerably affected by the location, phenophase and genotype in both experimental years (Tables 3 and 4). However, the different mineral nutrition and climate conditions at the two locations had an impact on nutrient assimilation and the results obtained were in accordance with many authors who emphasised the importance of N in wheat nutrition (Cox et al., 1985; Osaki et al., 1991; Frederick, 1997; Peltonen-Sainio et al., 1997; Waldern and Flowerdy, 1979; Pavlov, 1984; Kovtun, 1974). As the vegetation period advanced the dry matter content increased, as the result of increased photosynthesis. This was confirmed by the significant positive correlations between LAI and chlorophyll *a*, *b* and carotenoids, and by the negative correlations with SLA and dry matter, and there was also a negative correlation between the photosynthetic parameters investigated and the SLA (Tables 5 and 6).

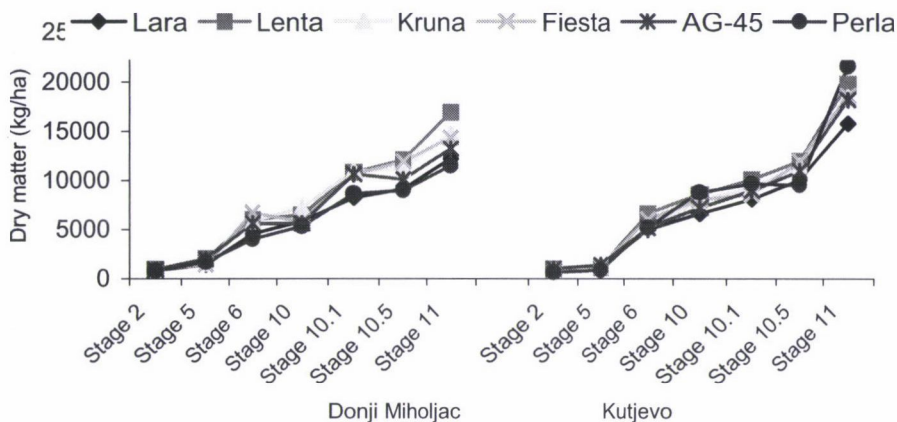


Figure 7. Average values of dry matter accumulation (kg/ha) of the investigated wheat genotypes per phenophase (stage) at both locations in 1997/98 and 1998/99

Statistical analyses showed a very significant correlation between dry matter content and the other parameters investigated (Tables 5 and 6), the influence of phenophase and location being most pronounced (Tables 3 and 4).

The chloroplast pigment content in the genotypes was affected by the phenophase and location, and a strong interaction was determined between the location, phenophase and genotype (Tables 3 and 4). The significance of this interaction can be explained by the different mineral nutrient supplies at the growing sites, especially the different nitrogen supplies. In both years the chloroplast pigment concentrations showed the highest positive correlation with LAI and dry matter accumulation and a negative correlation with SLA (Tables 5 and 6).

Table 5

Coefficients of correlation between the investigated parameters and the accumulation of dry matter in the aboveground plant organs at both locations in 1997/98 ($P < 0.05^*$; $P < 0.01^{**}$)

Parameters	Chl <i>b</i>	Carot.	LAI	SLA	Dry matter
Chl <i>a</i>	0.667**	0.925**	0.579**	-0.355**	0.837**
Chl <i>b</i>	—	0.761**	0.504**	-0.211 ^{NS}	0.594**
Carot.	—	—	0.534**	-0.290**	0.695**
LAI	—	—	—	-0.210**	-0.554**
SLA	—	—	—	—	-0.506**

*Significant at the 5% level, **significant at the 1% level, ^{NS} not significant

Table 6

Coefficients of correlation between the investigated parameters and the accumulation of dry matter in the aboveground plant organs at both locations in 1998/99 ($P < 0.05^*$; $P < 0.01^{**}$)

Parameters	Chl <i>b</i>	Carot.	LAI	SLA	Dry Matter.
Chl <i>a</i>	0.617**	0.825**	0.570**	-0.315**	0.887**
Chl <i>b</i>	—	0.661**	0.512**	-0.311 ^{NS}	0.604**
Carot.	—	—	0.503**	-0.240**	0.715**
LAI	—	—	—	-0.501**	-0.564**
SLA	—	—	—	—	-0.516**

*Significant at the 5% level, **significant at the 1% level, ^{NS} not significant

The relationship between dry matter accumulation and other physiological parameters, e.g. chloroplast pigment concentration, LAI and SLA (Tables 5 and 6), is stable and significant, but is strongly influenced by external factors (Tables 3 and 4). Phenophase, i.e. the dynamics of wheat growth and development, along with the interaction between location and phenophase, have the greatest influence on the photosynthetic parameters (chloroplast pigment content, leaf area, SLA and accumulation of dry matter). The impact of location and phenophase is greater than that of genotype for all the parameters investigated, with the exception of chlorophyll *a* and carotenoids (Tables 3 and 4). The interaction of location and phenophase indicates that the grain yield depended on the climatic growing conditions and the mineral nutrient supply. The genotypes examined showed statistically significant differences for all the photosynthetic parameters investigated (Tables 3 and 4). The differences between the investigated genotypes for both the biological and grain yields can be explained by differences in photosynthetic parameters. The new wheat genotypes responded differently under the specific agroecological conditions to a great number of factors which have a decisive role in yield performance. The link between the dry matter content and the other parameters examined was strong and significant, though external factors also had a strong influence.

Table 7

Average values of biological and agricultural yield (t/ha) for the genotypes investigated at Donji Miholjac in 1997/98 and Kutjevo in 1998/99

Genotypes	Donji Miholjac		Kutjevo	
	Biological yield	Agricultural yield	Biological yield	Agricultural yield
Lara	18.92	7.36	21.88	8.40
Lenta	23.55	9.20	25.37	9.70
Kruna	21.70	7.63	25.02	8.45
Fiesta	22.56	9.30	20.63	7.60
AG-45	19.54	7.92	23.30	8.30
Perla	23.82	9.80	21.80	8.10
Average	21.68	8.52	22.98	8.42

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SCREENING DROUGHT TOLERANCE CRITERIA IN MAIZE

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Six pure lines of maize were tested in a randomized complete block design with three replications under irrigated and rainfed conditions. Genetic variation was found between the genotypes for yield potential (Y_p), stress yield (Y_s), tolerance index (TOL), geometric mean productivity (GMP), harmonic mean (HM) and stress tolerance index (STI). Stress tolerance index was corrected using a correction coefficient (k_i) and thus a modified stress tolerance index (MSTI) was introduced as the optimal selection criterion for drought-tolerant genotypes. The results of three-D plotting indicated that the most desirable genotype for irrigated and rainfed conditions was the genotype K1515, for non-stressed conditions K18 and for stress conditions K104/3, K760/7 and K126/10.

Key words: maize, drought tolerance, biplot, modified stress tolerance index

Introduction

The improvement of drought tolerance has been defined as a desirable breeding objective in crops such as maize (Clark et al., 1992). Drought tolerance in native plant species is often defined as survival, but in crop species it should be defined in terms of productivity (Passioura, 1983). The definition of drought tolerance as the ability of plants to grow satisfactorily when exposed to water deficits has little direct applicability to either quantifying or breeding for the character in crop species (Clark et al., 1992).

In the absence of an understanding of the special mechanisms of tolerance the quantification of drought tolerance should be based on the grain yield under dry conditions (Fischer and Maurer, 1978). It is worthwhile, therefore, to look at the methods that have been used to quantify tolerance. Several selection criteria are proposed to select genotypes based on their performance in stress and non-stress environments (Fernandez, 1992). Rosielle and Hamblin (1981) defined stress tolerance as the difference between grain yield in stress (Y_s) and non-stress (Y_p) environments, and mean productivity (MP) as the average of Y_p and Y_s . Fischer and Maurer (1978) proposed a stress susceptibility index (SSI). Fernandez (1992) defined a new stress tolerance index (STI).

Genotypes can be categorized into four groups based on their performance in stress and non-stress environments: genotypes which express uniform superiority in both stress and non-stress environments (Group A); genotypes which perform favourably only in non-stress environments (Group B); genotypes which yield relatively well only in stress environments (Group C) and genotypes which perform poorly in both stress and non-stress environments (Group D). The optimal selection criteria should distinguish Group A from the other three groups (Fernandez, 1992).

The objectives of the current experiment, carried out in the Agricultural Research Station of Dezful, Iran in the year 2000, were (i) the screening of quantitative criteria of drought tolerance, (ii) the introduction of a new drought tolerance index and (iii) the identification of drought-tolerant genotypes.

Materials and methods

Six pure lines of maize, namely K104/3 (1), K760/7 (2), K1515 (3), K18 (4), K19 (5) and K126/1 (6), were cultivated in a randomized complete block design with three replications under two different environments (irrigated and rainfed) in the Agricultural Research Station of Dezful, Iran. From each pure line 50 seeds were selected and single seeds were sown in 5 m rows with 20×75 cm plant to plant and row to row distances, respectively. The minimum and maximum temperatures at the station were 5.6°C and 54.6°C , respectively. The average rainfall in 2000 was 250 mm and the region was arid. The chemical properties of the soil in the experiment were reported as:

Soil properties	Value
E.C.	0.84 dS/m
pH	7.87
O.C.	0.48%
Mn	2.55 ppm
P	6.7 ppm
K	101 ppm
Cu	1.31 ppm
Fe	3.17 ppm
Zn	0.32 ppm

Each plot consisted of 4 rows, the two middle rows being planted with the tested genotypes and the two lateral rows with the genotype SC 704 to eliminate the border effect. Ten competitive plants were randomly selected from each entry in both the irrigated and rainfed treatments and the yield potential (Y_p) and stress yield (Y_s) were recorded. Using Y_p and Y_s the following quantitative indices of drought tolerance were calculated:

Tolerance index (TOL) and mean productivity (MP) (Rosielle and Hamblin, 1981):

$$\text{TOL} = (Y_p - Y_s) \text{ and } \text{MP} = \frac{Y_p + Y_s}{2}$$

Harmonic mean (HM) (Zahravi, 1999):

$$\text{HM} = \frac{2(Y_p \times Y_s)}{Y_p + Y_s}$$

Stress susceptibility index (SSI) (Fischer and Maurer, 1978):

$$\text{SSI} = \frac{1 - (Y_s/Y_p)}{\text{SI}}; \text{SI} = 1 - (Y_s/Y_p)$$

where SI is stress intensity and Y_s and Y_p are the means of all genotypes under stress and irrigated conditions, respectively.

Geometric mean productivity (GMP) and stress tolerance index (STI) (Fernandez, 1992; Kristin et al., 1997):

$$\text{GMP} = \sqrt{(Y_p)(Y_s)}, \text{ STI} = \frac{(Y_s) \times (Y_p)}{(\bar{Y}_p)^2}$$

Modified stress tolerance index (MSTI):

$$\text{MSTI} = k_i \text{ STI}, k_1 = \frac{Y_p^2}{Y_d^2} \text{ and } k_2 = \frac{Y_s^2}{Y_s^2}$$

where k_i is the correction coefficient.

Analysis of variance, mean comparison, correlation analysis and three-dimensional plotting were done using the MSTAT-C and SPSS statistical softwares.

Results and discussion

The results of analysis of variance (Table 1) for various quantitative criteria of drought tolerance showed highly significant differences for all the indices except SSI, indicating the presence of genetic variation and the possibility of selection for drought-tolerant genotypes based on Y_p , Y_s , TOL, GMP, HM and STI.

Genetic variation between maize genotypes was reported for yield by Bolanos and Edmeades (1996) and Morris et al. (1991), for drought resistance by Vasal et al. (1997) and Banziger et al. (1997) and for Y_p , Y_s , TOL, MP, GMP, SSI, HM and STI by Ahmadzadeh (1997) and Afarinesh (2000).

The estimates of stress tolerance attributes (Table 2) indicated that the identification of drought-tolerant genotypes based on a single criterion was contradictory. For example, according to TOL, the desirable drought-tolerant genotype was K104/3 (1), while according to STI the most desirable drought-tolerant line was K1515 (3). Moreover, MP failed to distinguish the group A and group B genotypes, while TOL and SSI failed to distinguish between group C and group A (Fernandez, 1992). To determine the most desirable drought tolerance criteria, the correlation coefficient between Y_p , Y_s and quantitative indices of drought tolerance was calculated (Table 2).

Table 1

Analysis of variance for different indices of drought tolerance under irrigated and rainfed conditions

Source of variation	d.f.	Mean square							
		Y_p	Y_s	TOL	MP	GMP	HM	SSI	STI
Genotypes	5	5480**	3382**	766**	4232**	4145**	4262**	0.60	0.83**
Replication	2	534*	651	1421*	26.18	11.07	68.9	0.79	0.03
Error	10	127*	418.8	415.4	70.70	76.90	122.9	0.38	0.03

*, ** Significant at the 5% and 1% probability level, respectively.

Table 2

Estimates of stress tolerance attributes from potential yield and stress yield data for maize genotypes

Lines	Y_p	Y_s	TOL	MP	GMP	HM	SSI	STI	k_1 STI	k_2 STI
K104/3	51.9	48.4	3.6	50.2	50.2	50.1	0.27	0.19	9.4	11.8
K760/7	52.9	48.9	3.9	50.9	50.8	50.8	0.31	0.19	9.9	12.3
K1515	78.4	42.9	25.7	65.5	64.1	62.8	1.32	0.30	34.7	22.6
K18	50.3	14.2	20.9	32.7	26.7	22.1	1.09	0.05	2.5	0.29
K19	61.5	31.6	29.8	46.5	44.8	41.4	1.9	0.14	10.2	3.91
K126	33.9	31.2	6.2	32.8	32.5	32.6	0.65	0.07	1.5	1.83

Table 3 showed that MP and STI had a positive significant correlation with Y_p and Y_s ; thus, MP and STI were better predictors of mean Y_p and mean Y_s than the other indices. However, MP fails to distinguish between group A and group B, while STI is estimated based on GMP; the rank correlation between STI and GMP is thus equal to 1 (Rosielle and Hamblin, 1981; Fernandez, 1992). The higher the value of STI for a genotype, the higher its stress tolerance and yield potential. The stress intensity value is also incorporated in the estimation of STI. Therefore, STI is expected to distinguish group A from group B and group C. This result was in close agreement with the findings of Fernandez (1992), Maroufi (1998), Imamjomah (1999) and Farshadfar et al. (2001).

In the STI index, Y_p^2 is a constant value, while the square root of the multiplication of Y_p and Y_s is the geometric mean of a genotype under stress and non-stress conditions. For this reason a pair of numbers with different natures may have the same geometric mean. For example, the geometric mean for the data pairs 1 and 12, 2 and 6, and 3 and 4 is 3.46, while these data, if related to the yield of the genotypes, have clearly different natures. This problem arises in the stress tolerance index (STI) and hence decreases its efficiency in distinguishing group A genotypes from the other groups (Naderi et al., 1999).

To improve the efficiency of STI a modified stress tolerance index (MSTI) was calculated as k_i STI, where k_i is a correction coefficient which corrects the STI as a weight. Therefore, k_1 STI and k_2 STI are the optimal selection indices for stress and non-stress conditions, respectively. Considering Y_p and Y_s as dependent and k_1 STI, k_2 STI and STI as independent variables, the contribution of k_1 STI to Y_p in relation to STI was $R^2=0.817$, while the contribution of STI to Y_p was $R^2=0.65$. The contribution of k_2 STI to Y_s was $R^2=0.78$, while that of STI was $R^2=0.72$. Thus, k_1 STI and k_2 STI are better predictors of Y_p and Y_s , respectively, in non-stress environments.

Table 3
Correlation coefficients between Y_p , Y_s and drought tolerance indices

	Y_p	Y_s	TOL	MP	GMP	HM	SSI	STI	k_1 STI	k_2 STI
Y_p	1	0.46	0.65	0.85*	0.77	0.69	0.50	0.82*	0.90*	0.74
Y_s	—	1	-0.29	0.86*	0.92*	0.96**	-0.34	0.88*	0.66	0.88*
TOL	—	—	1	0.20	0.10	-0.07	0.96**	0.15	0.42	0.03
MP	—	—	—	1	0.99*	0.97**	0.086	0.99**	0.92*	0.95**
GMP	—	—	—	—	1	0.99**	0.014	0.99**	0.87*	0.95**
HM	—	—	—	—	—	1	-0.08	0.97**	0.83*	0.94**
SSI	—	—	—	—	—	—	1	0.034	0.28	-0.12
STI	—	—	—	—	—	—	—	1	0.92**	0.97**
k_1 STI	—	—	—	—	—	—	—	—	1	0.90*
k_2 STI	—	—	—	—	—	—	—	—	—	1

*, ** Significant at the 5% and 1% levels of probability, respectively.

Using STI, k_1 STI and k_2 STI as the optimal selection criteria the most desirable genotype for irrigated and rainfed conditions was K1515 (STI=0.30, k_1 STI=34.7 and k_2 STI=22.6). A three-dimensional plot between Y_p , Y_s and STI (Fig. 1) was used to distinguish the group A genotypes from the other three groups (B, C and D) (Fernandez, 1992; Farshadfar et al., 2001). In this case the most desirable genotype for irrigated and rainfed conditions was K1515, for non-stress conditions K18 and for stress conditions K104/3, K760/7 and K1264/1.

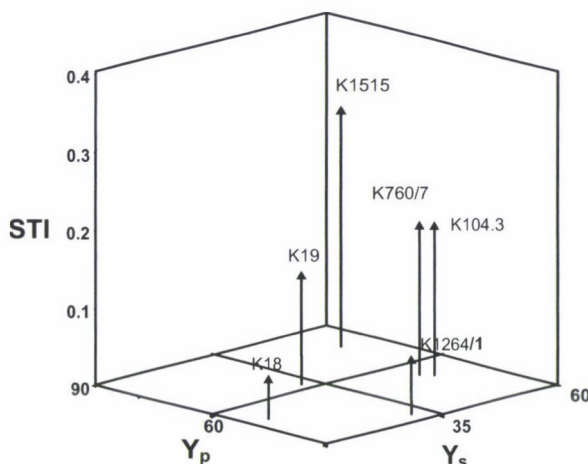


Fig. 1. Three-dimensional plot between Y_p , Y_s and STI

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SEED YIELD OF SUGAR BEET AS AFFECTED BY STAND DENSITY AND HARVESTING DATE

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A three-year field trial was conducted to study the effect of plant population and harvesting dates on the yield of cleaned 2.0–6.0 mm seed and the seed yield (g) per plant.

The highest seed yield was obtained with a spacing of 50 × 12 cm, or 160,000 plants/ha. A decrease in the plant-to-plant spacing to 9 cm decreased the yield by an average of 70 kg/ha over the three study years. The seed yield decreased to an even greater extent when the plant-to-plant spacing was 16 or 24 cm.

The seed yields increased the most between the first and second harvesting dates: 400 kg/ha, or 50 kg/ha a day. On the last harvesting date, the seed yield was as low as 5–8 kg/ha. The yield loss was somewhat higher in the most densely sown treatment. The effect of spacing and harvesting date on seed yield per plant was similar to that on total seed yield.

Key words: seed yield, harvesting date, vegetative space, stand density

Introduction

Although in practice beet seed yields most often range between 2.0–2.5 t/ha, considerably higher yields of this crop have sometimes been reported. Senff and Scminkel (1988), for example, reported yields of 3.00 t/ha in Germany and the U.S.A., while Radišić (1987) also obtained high yields in trials, albeit with multigerm selection components. Kristek and Matić (1984) argued that 65,000–80,000 plants per hectare was enough to secure high yields under agroecological conditions similar to those described by Radišić (1987). In a study on the impact of row-to-row spacing, Longden and Scott (1973) obtained a yield 370 kg/ha higher with rows spaced at 25 cm than when the spacing was 50 cm. In the more densely spaced treatment (25 × 15 cm), the yield increased up to 257,000 plants/ha, while in the less dense spacing (50 × 5.2 cm), the yield increased up to 385,000 plants/ha. This shows the great significance of competition not only among the plants within the rows but also among the plants in neighbouring rows. Radišić (1987) studied stand densities from 50,000 to 500,000 plants/ha using multigerm materials and obtained the highest yield with 100,000 plants/ha (50 × 20 cm). This suggests that under the agroecological conditions in Yugoslavia considerably fewer plants per hectare are needed for maximum yields compared with the plant populations recommended for the conditions in Northern Europe. In England, according to Scott (1968), it rains every third day on average at the time when seed beet is flowering. In a review of seed beet production in North America, Campbell (1968) reported the annual precipitation in the region with the largest production to be 1,000 mm.

Longden (1972) assumed that the lack of a more definitive effect of the harvesting date was due to differences in the maturity of crops at the same calendar dates. In each year of the study, he carried out harvesting on certain dates. In the wet year, the yield increased until the middle harvesting date, whereas in the dry one the yield began to decrease as early as the first harvesting date. The author concluded that this was due solely to differences in the maturity of the crops.

Comparing different growing methods, Senff and Scminkel (1988) obtained 15,000 seeds per plant when the plants were grown from stecklings and only 1,000 seeds per plant when using a one-year method of production.

Materials and methods

A field trial with the three-way cross hybrid Dana (normal type) was carried out in 1998/1999, 1999/2000 and 2000/2001 using a one-year method of production. Factor A, the harvesting date, included the following treatments: 30, 38, 45 and 50 days after full flowering, Factor B was the year and Factor C the stand density, consisting of the following treatments: 50×9 cm, 50×12 cm, 50×16 cm and 50×24 cm. The basic plot size was 10 m^2 , with elongated plots and a randomized block design.

The aim of the paper was to determine the optimal number of seed plants per unit area and the best time for harvesting.

Results

Analysis of variance showed a very significant effect of the harvesting date, stand density, ecological conditions, year, and interaction between harvesting date and year on the sugar beet seed yield (Table 1). Similar results were reported by Kristek and Matić (1984).

All the stand densities studied except the thinnest one increased the seed yield of sugar beet. The rates of yield increase in the denser treatments were all very similar. Between the first and second harvest date, the seed yield increased by 400 kg/ha, or 50 kg/ha a day (Fig. 1). During the week that followed, the yield decreased by 300 kg/ha (40 kg/ha per day) on average. In the most densely spaced treatment, the drop was the smallest (200 kg/ha). On the last harvesting date, the reduction in seed yield was small, only 5–8 kg/ha. A somewhat bigger decrease was recorded in the densest treatment. At all the densities, the maximum yields were measured on the second harvesting date. The difference between the highest yield, obtained with 50×12 cm, and the lowest one, obtained with 50×24 cm, was 200 kg/ha.

The effect of plant-to-plant spacing on the seed yield was less than 10%. At all the harvesting dates, the highest yields were obtained with a spacing of 50×12 cm, followed by 50×9 cm, 50×16 cm and 50×24 cm. Therefore, an increase in spacing from 9 to 12 cm increased the total seed yield. Any increase in spacing above 12 cm, however, decreased the seed yield. A higher plant

population, 160,000–222,000/ha, produced a higher seed yield, and vice versa, a smaller population, 83,000–125,000/ha, gave a lower yield. The differences in yield between the two denser and two thinner stands were therefore significant. Although the stand density had a definite influence on yield, it is worth noting that even the smallest plant population in this study produced good seed yields. As little as 83,000 properly spaced plants per hectare were enough to secure a good yield of seed. This is indicative of the extraordinary ability of the plants to take advantage of the extra space provided by a thin stand; that is to say, to compensate for the lower number of plants per hectare by producing higher yields.

The effect of spacing and harvesting date on seed yield per plant was similar to that of the two factors on total seed yield. The seed yields depended on the size of the vegetative space the plants were grown on. During the period of maximum yields, at the second harvesting date, plants that were 9 cm apart had 13.6 g of seed, those spaced at 12 cm had 16.4 g, while those separated by 16 or 24 cm produced 21.5 and 32.8 g, respectively (Fig. 2). The greatest “yield lag” relative to the most densely spaced plants was recorded in plants grown 24 cm apart.

Table 1
Analysis of variance for sugar beet seed yield

Sources of variation	Degrees of freedom	Mean square	F-Value
Harvesting date (A)	3	1.01	18.77**
Year (B)	2	0.34	6.31**
A × B	6	1.46	27.27**
Stand density (C)	3	0.31	5.84**
A × C	9	0.02	0.29 ns
B × C	6	0.04	0.75 ns
A × B × C	18	0.03	0.59 ns
Error	96	0.05	—
Total	143	—	—

*, **: significant at the 5% and 1% probability levels, respectively; ns: non significant

Discussion

At the first harvesting date, the differences in fruit moisture content between the study years were small, since the fruits were at the start of the ripening process. The average dry matter content was only 23.1%. Senff and Schminkel (1988) reported a threshold value of 60% for fruit dry matter content and found that if sugar beet plants were harvested before the fruits reached this dry matter percentage, seed viability was poorer. Šroller (1984) also concluded that harvesting should be done when the fruits contained 58–60% dry matter. The postponement of harvesting beyond this date did not improve seed viability. In the present study, the fruit dry matter content did not reach the 60% threshold before the third harvesting date in any of the study years.

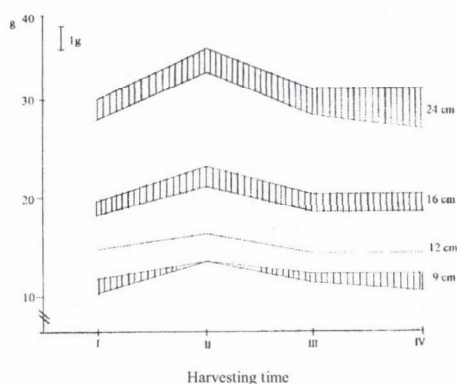


Fig. 1. Seed yield/plant (three-year average)

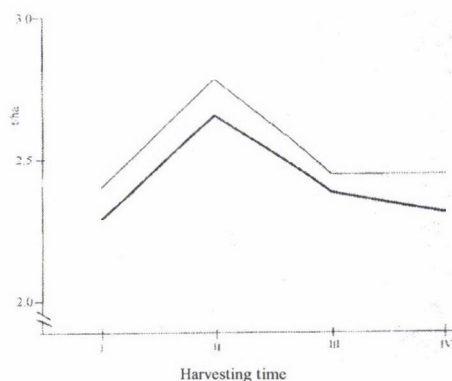


Fig. 2. Yield of cleaned seed

Many researchers have found a high water content in the pericarps of physiologically mature fruits. Senff and Schminkel (1988) and McFarlane (1975) determined physiologically mature fruits to be 70 and 50% water, respectively, while Snayder (1971) reported that the fruits contained 62–77% water 40 days after the appearance of the first flower. All these authors stressed the importance of varietal characteristics: it was observed that monogerm hybrids, especially hybrids resistant to bolting, had a longer growing season, so that in some years and some regions problems occurred at the time of threshing because conditions were not favourable for the drying of the seeds.

In all the study years, the daily loss of fruit water was smaller during the first part of the growing season, i.e. until full seed maturity. After that, the daily fruit water loss increased: between the second and third harvesting dates 1.5% of water was lost each day, while between the third and the fourth the loss was 3%. These findings are in contradiction with those of Snayder (1971), where the loss of moisture at the beginning of ripening was found to be as much as 45%, or about 8.5% a day. When the moisture content dropped below that level, the daily loss was smaller, around 3.6%. Snayder (1971) obtained these data under controlled conditions at 17.8°C with 5% relative humidity. Comparisons between the two studies are hindered to a large extent by the great polymorphism of sugar beet plants and by the lack of a specific criterion that could be used to determine the period under observation for different conditions and years relative to the stage of organogenesis, phase of maturity, etc.

The impact of plant population on the seed yield is clear, although the differences between the treatments were not large. The highest yields were obtained with a spacing of 50 × 12 cm, or 160,000 plants per hectare. A decrease in the plant-to-plant spacing to 9 cm reduced the yield by 70 kg/ha (three-year average). The seed yield decreased much more when the plant-to-plant spacing was increased to 16 or 24 cm. A possible explanation for this could be that the 4 cm increase in spacing (from 12 to 16 cm) was not large enough to have a

significant effect on the morphological changes in the plants, so they could not compensate substantially for the deficit of 40,000 plants/ha. However, a further 8 cm increase in the spacing gave the plants sufficient room for more intensive growth and increased branching, allowing them to compensate more successfully for the insufficient number of plants. In the U.S.A., researchers have studied much denser stands than in Europe. On the basis of trial results, Cooke and Scott (1993) recommended 600–900,000 plants/ha before the winter and 350,000 plants/ha at harvesting. Longden and Scott (1973) recommended 300,000 plants/ha after processing data from a number of experiments, in some of which the yield increased up to 385,000 plants/ha. Under the production conditions in Yugoslavia, the plant population rarely exceeds 150,000 plants/ha, and the row-to-row spacing is 50–60 cm. Based on their trial results, Kristek and Matić (1984) concluded that sowing should be done so as to achieve 160,000–200,000 plants/ha at emergence and that 65,000–80,000 plants/ha at harvest was enough to attain good yields. Halvaček (1978) and Montanari et al. (1982) studied the effects of thinner stands of two, three or four plants per metre and found that the highest yields were achieved with the largest number of plants.

Many researchers emphasize the importance of plant uniformity, less lushness in growth and earlier maturity, all of which, according to their results, can be achieved only in very dense stands. It should be stressed, however, that agroecological conditions alone could not bring about such large differences in the optimum stand density if it were not for the ability of sugar beet plants to utilize increased vegetative space very successfully. Sugar beets achieve this ability thanks to highly intensive growth and polymorphism, which are characteristic of the species. In the second year of growth, once they have undergone vernalization, sugar beets lose their capability for unlimited growth, since by that time the plant processes are directed towards the formation of generative organs and the completion of vegetative growth, which is still pronounced, primarily due to the fact that the formation of new flowers lasts almost until the end of the growing season. This provides polymorphism regarding the degree of fruit maturity. In other words, polymorphism, which is of great importance for the species, is the result of more pronounced vegetative growth in the second year of cultivation.

When determining the dates of harvesting, it is important to bear in mind that the harvest should be delayed for as long as seed yield losses do not outweigh the gain from increased seed viability. Studying the maturation stage, Scott and Longden (1973) established that under English conditions sugar beet seed reached maximum viability two weeks after reaching maximum yield. Longden et al. (1974) argued that seed yield losses of 5–10% could be tolerated if seed viability increased fast enough. In the present study, the yield increased until the second harvesting date. The average increase during the three study years was 15%, or about 50 kg/ha/day.

Seed yield losses were the same regardless of the spacing, i.e. the results of the study do not indicate that more densely sown crops mature earlier or that they should be harvested earlier. There was mostly no interaction between the spacing and the harvesting date with regard to yield. Senff and Scminkel (1988) argued that every treatment had its own optimal harvesting date. They reported that in their study earlier sowing caused earlier maturity and greater seed losses.

Seed yield per plant directly affects the total yield per unit area, so the treatments in the present study had the same effect on both these indicators of yield. Comparing different growing methods, Senff and Scminkel (1988), reported obtaining as many as 15,000 seeds from stands with 500,000–700,000 plants/ha. Kristek and Matic (1984) also reported large differences in seed yield per plant: 218 g/plant at 5,200 plants/ha compared with 65 g/plant at 51,000 plants/ha.

These data show that seed sugar beet is capable of producing a three to four times higher seed yield per plant in thinner stands. This ability is somewhat higher in sugar beet than in other field crops because of its intensive vegetative growth almost until the very end of the growing season. The harvesting date should be carefully chosen because of the very significant effect of ecological conditions, i.e. the year.

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EFFECT AND RESIDUAL EFFECT OF N FERTILISATION ON THE N UPTAKE OF WINTER WHEAT IN A LONG-TERM FIELD EXPERIMENT

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In wheat production, N fertilisation is one of the most effective agro-technical devices to increase yield and N concentration. In Hungary, fertiliser use, particularly that of N, has dropped dramatically in the last decade. The aim of this experiment was to study the direct and residual effect of N fertilisation on the grain yield and N uptake of winter wheat after 30 years of intensive N fertilisation. A long-term fertilisation experiment was set up on brown forest soil (Eutric Cambisol) with medium N status at Keszthely (Hungary) in 1965. In 1995, the plots were halved. From that year on, half of the plots no longer received N fertiliser, while the other half of the plots was fertilised with increasing N doses. Two years after the treatment modification, no residual effect of long-term intensive N fertilisation (10.44 t N/30 years) could be detected. Under the investigated site conditions, the omission of yearly N fertilisation led to low wheat yields and low N concentrations both in the grains and in the vegetative organs above the uppermost internode.

Key words: long-term field experiment, N fertilisation, N uptake, residual effect, winter wheat

Introduction

Nitrogen is the most important macronutrient determining wheat grain yield and quality. The results of numerous field experiments conducted in Hungary under widely different agroecological conditions have shown that Hungarian-bred modern cultivars could not achieve high grain yields of good quality without the application of appropriate amounts of N fertiliser.

Wheat plants suffering from N deficiency have a poor growth rate and a shorter vegetative growth stage (Lönhard-Bory et al., 1995), which negatively influences the assimilation area. Many results have proved a close correlation between the grain yield and leaf area of wheat (Lönhard-Bory and Ragasits, 1990; Lönhard-Bory and Kismányoky, 1992). On the other hand, nitrogen over-fertilisation may lead to yield depression and has serious environmental impacts.

A considerable part of the nitrogen accumulated in the vegetative plant parts before flowering is reutilised in the developing wheat grains through translocation (Simpson et al., 1983; Clarke et al., 1990; Berecz et al., 1997). Nitrogen fertilisation may considerably increase both the amount available for redistribution and the extent of nitrogen translocation from the vegetative parts into the grains (Spiertz and Ellen, 1978; Papakosta and Gagianas, 1991).

In Hungary, N fertiliser use has dropped dramatically in the last decade. According to statistical data, N fertiliser expenditure averaged 550 and 177 thousand t N/year in 1971–1990 and 1991–1996, respectively. The decline in fertiliser consumption can be partly attributed to the greatly increased fertiliser prices.

The aim of this experiment was to study the direct and residual effects of N fertilisation on the grain yield and N uptake of various upper winter wheat parts after many years of intensive N fertilisation. Long-term fertilisation experiments, which have long-term balanced soil nutrient levels, provide a reliable basis for studying the nutrient responses of crops.

Materials and methods

Long-term N fertilisation experiments were set up on brown forest soil (Eutric Cambisol) at Keszthely (Hungary) in 1965. The treatments included increasing nitrogen doses. In addition to the nitrogen doses, each plot received 140 kg/ha each of P_2O_5 and K_2O before ploughing in autumn. The trials were done on plots of 96 m², in a randomised block design, in six replications, with a two-year crop rotation of maize – winter wheat. After 30 years, in 1995, the plots were halved and the number of replications was reduced to three. From that year on, half of the plots received no further N fertiliser, while the other half was fertilised with 100, 150 or 200 kg N/ha. These changes in the experimental plan provided the opportunity to study the residual and direct effects of different N doses. In 1998, five of the 24 different N treatments were chosen (Table 1) to investigate these effects on the nitrogen uptake of winter wheat (Mv 23).

The original fertility of this nearly neutral sandy loam soil was medium for nitrogen and potassium and poor for phosphorus (Table 2). No significant changes could be detected in the humus content of the control plots after long-term fertilisation with 348 kg N/ha/year. Precipitation was 727, 534 and 297 mm in 1996, 1997, and from January till harvest in 1998, respectively. A detailed description of the soil and climatic characteristics of the site is available in the work of Debreczeni (1994).

Table 1
Treatments applied in the experiment (N, kg/ha/year)

Treatment No.	1965–1995	Since crop year 1995/96	
		Before ploughing	Early spring
1	–	–	–
2	348	–	–
3	348	50	50
4	348	75	75
5	348	100	100

Table 2
Main chemical soil characteristics of the experimental field (1965)

Soil characteristic	
pH (H ₂ O)	7.5
pH (KCl)	7.0
AL- P_2O_5 (mg/kg)	15
AL- K_2O (mg/kg)	130
Total N (%)	0.13
Humus (%)	1.7

AL: Ammonium lactate-extractable

Between the grain developmental stage with 66.3–70.1% moisture to full ripening, six harvests were made (Table 3). On each occasion, 30 plants per plot were cut. The flag-leaves and uppermost internodes were removed from the plants, the spikes were hand-threshed and the glumes and rachis were separated. The samples were air-dried, then the weight of the plant parts was determined. After grinding the samples, the N concentration of the plant parts was measured by Kjeldahl's modified method (Lengerken et al., 1974).

Table 3
Grain moisture (%) pertaining to the different sampling dates in Treatments 1–5

June 8	June 11	June 16	June 23	June 30	July 7
66.3–70.1	60.9–65.8	54.1–58.8	46.9–48.7	32.0–36.2	17.4–18.0

Results and discussion

Two years after the treatment modification, no significant differences could be detected between the grain yield, thousand-grain mass and grain N yield of plots receiving no N fertiliser at all and those previously fertilised with 348 kg N/ha/year for 30 years (Table 4). Compared to the above treatments, N fertiliser doses of 100–200 kg/ha significantly increased the grain yield and grain N yield. The highest grain yield could be achieved even with 100 kg N/ha; the yield increases achieved with higher N doses were not statistically significant. In the Keszthely region, 120–140 kg N/ha is necessary to obtain the highest yields (Ragasits, 1980; Berecz, 1989). The grain yield surplus per 1 kg N fertiliser was also the highest with the application of 100 kg N/ha (Fig. 1).

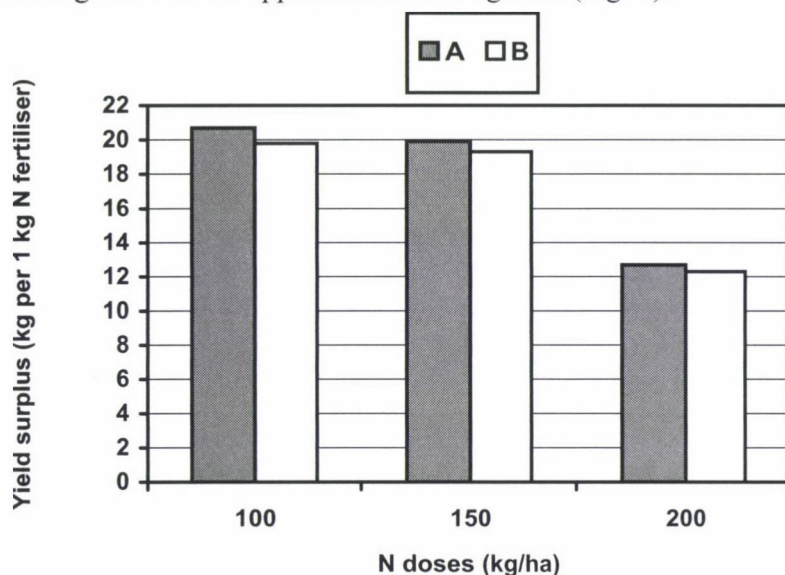


Fig. 1. Grain yield surpluses as compared to Treatments 1 (A) and 2 (B)

Table 4
Grain yields, thousand-grain masses and grain N yields in the different treatments

Treatment No.	Grain yield (t/ha)	Thousand-grain mass (g)	Grain N yield (kg/ha)
1	1.65	38.2	28.6
2	1.74	40.8	34.3
3	3.72	43.0	67.8
4	4.64	44.7	94.9
5	4.19	44.5	88.2
LSD _{5%}	1.50	3.4	35.2

No significant differences could be detected in the N content of the tested vegetative plant parts from plots receiving no N fertiliser at all and those previously fertilised with 348 kg N/ha/year for 30 years at any of the sampling dates (Table 5). Compared to these treatments, fertilisation with 100–200 kg N/ha significantly increased the amount of N accumulated in the vegetative plant parts before grain filling. In the ripe grains per ear, the highest N amounts were measured after the application of 150 kg N/ha. A further increase in the N doses resulted in no further increase in the N content of the grains. In the Keszthely region, 160 kg N/ha was necessary to reach the highest grain N concentrations in N fertilisation experiments carried out by Berecz and Ragasits (1990).

The vegetative N contents showed various rates of decrease during grain development. In accordance with the findings of several authors (Spiertz and Ellen, 1978; Waters et al., 1980), these results indicate that a significant part of the nitrogen demand of the developing grains may have been covered by the translocation of the nitrogen assimilates accumulated in these vegetative organs.

The incorporation of N into the grains lasted up to the fifth samplings when 32–36% grain moisture contents were measured. The N depletion from the vegetative plant parts during this time could be well described by linear regressions. Tables 6–8 show the results only for Treatment 3, which caused the highest rate of N depletion from all the tested vegetative parts. Similarly to previous findings (Berecz et al., 1997), the flag-leaves showed the highest rate of nitrogen depletion (Table 6). This plant part may have contributed to the grain N to the highest degree in all the treatments. The N amounts mobilised from the four tested vegetative parts amounted to 20% of the N content of the ripe grains per ear after treatment with 100 kg N/ha (Table 8). The results suggest that the glumes could be the second major contributor to the grain N. Simpson et al. (1983), Harms and Nowak (1990) and Berecz et al. (1997) found that the glumes were important sources of N translocation to the grain.

Table 5
Nitrogen content of the wheat plant parts at different sampling dates

Treatment No.	Sampling dates					
	June 8	June 11	June 16	June 23	June 30	June 7
Uppermost internode (mg N/30 plant parts)						
1	45	36	27	29	14	16
2	48	51	34	28	22	21
3	73	61	57	46	33	28
4	84	78	54	60	55	43
5	90	85	64	72	55	47
LSD _{5%}	14	20	20	20	14	11
Flag-leaf (mg N/30 plant parts)						
1	67	57	64	47	19	13
2	67	75	55	40	23	16
3	121	90	90	55	36	22
4	135	115	90	113	92	44
5	150	140	109	138	110	41
LSD _{5%}	17	41	31	32	30	24
Glumes (mg N/30 ears)						
1	74	51	45	44	25	24
2	75	61	53	56	38	29
3	100	90	70	67	50	50
4	112	98	78	82	60	66
5	128	96	76	83	72	61
LSD _{5%}	22	16	18	11	14	14
Rachis (mg N/30 ears)						
1	13	11	9	8	7	8
2	14	14	11	11	9	9
3	18	18	15	15	12	13
4	20	19	17	20	18	18
5	22	20	17	17	19	17
LSD _{5%}	3	4	3	5	4	4
Grains (mg N/30 ears)						
1	127	163	258	403	456	485
2	218	369	299	545	651	587
3	314	409	482	748	828	819
4	286	379	488	883	1127	1015
5	311	515	549	887	1180	1016
LSD _{5%}	80	73	142	180	203	112

Table 6
Regression analysis results for N contents detected in upper vegetative plant parts in Treatment 3 during the period between 66.3 and 32.0% grain moisture ($y = a + bx$, $n = 15$)

Plant parts	A	b	r
Uppermost internode	71.45	-1.653	0.9189***
Flag-leaf	116.52	-3.590	-0.8910***
Glumes	97.89	-2.125	-0.9307***
Rachis	18.31	-0.240	-0.7786***

***P = 0.1%

Table 7

Relative decrease (%) in N content of the upper vegetative plant parts calculated from the values of regression lines (Treatment 3)

Uppermost internode	Flag-leaf	Glumes	Rachis	Sum of plant parts
52.1	69.9	29.2	48.8	56.5

Table 8

Percentage of N depleted from the upper vegetative plant parts per shoot in the nitrogen contained in the ripe grains per ear (Treatment 3)

Uppermost internode	Flag-leaf	Glumes	Rachis	Sum of plant parts
4.40	9.65	5.71	0.64	20.4

Conclusions

The results prove that the residual effect of 30 years of intensive N fertilisation practically ceased after two years. Under the given site conditions, it is necessary to apply N fertiliser yearly. The omission of regular N supplies leads to low wheat yields and N concentrations. Investigations into the N content of the vegetative plant parts during grain development confirmed the above findings.

Acknowledgements

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NITROGENOUS GAS PRODUCTION IN THE SOIL AIR AS AFFECTED BY DIFFERENT N FERTILISER FORMS AND WATER SUPPLIES IN MODEL EXPERIMENTS

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Large-pot model experiments were conducted with maize under greenhouse conditions with the aim of studying the effect of different N fertiliser forms, water supplies and crop residues on the nitrogenous gas production in the rooting zone. Nitrogen fertiliser was applied in the form of KNO_3 or NH_4Cl . The experiment was done at two soil moisture levels, with or without the incorporation of maize crop residues into the soil, and with or without test plants. Gas traps were placed in the pots at a soil depth of 20 cm. During the growing season, the trapped soil air was analysed for NO_x , N_2O and N_2 . Practically the same N amounts evolved in the soil air with both chemical forms of N fertiliser at both soil moisture levels. Expressed as a percentage of fertiliser N, the total amount of gaseous N evolved averaged 12.8% and 12.9% in the planted, and 23.8% and 24.3% in the unplanted pots with KNO_3 and NH_4Cl fertiliser, respectively. Higher soil moisture and the incorporation of crop residues resulted in higher NO_x -N and N_2O -N ratios within the total gaseous N evolved in the rooting zone.

Key words: maize, nitrogenous gases, N fertilisation, pot experiment, soil moisture

Introduction

For several decades there has been interest in the different processes causing N loss from N fertilisers. The main pathways for N loss from fertilisers are nitrate leaching, nitrogen in runoff water, ammonia volatilisation and the emission of gaseous nitrogen compounds. Similarly to mineral soil N, fertiliser N is also exposed to different transformation processes in the soil. As a result of these microbial processes (ammonification, nitrification, denitrification), part of the fertiliser N may escape from the soil in the form of various gaseous N compounds before being taken up by plants. Nitrogen fertilisation also increases the denitrification losses of mineral soil N because it increases the mineral N content available as substrate for soil microbial processes (Castaldi and Smith, 1998).

The gaseous N emission from the soil is of great importance from the environmental point of view. Nitrous oxide (N_2O) contributes to global warming and to the destruction of the ozone shield when it is decomposed to nitric oxide (NO) in the stratosphere. Nitrous oxide causes 5–6% of the present greenhouse effect (Laegreid et al., 1999). Agricultural soils emit 3.3 Mt N_2O -N yearly (Mosier et al., 1998). The deposition of NO and nitrogen dioxide (NO_2) in the form of rain and dust contributes to the acidification of soils and surface waters. It is estimated that about 0.5% of the applied fertiliser N is emitted in the form of NO (Veldkamp and Keller, 1997).

The rate and composition of nitrogenous gas production depend on many factors, e.g. on soil temperature and moisture (Mahmood et al., 1998), on the chemical form of N in the fertiliser (Berecz et al., 2000; Castaldi and Smith, 1998; Debreczeni and Berecz, 1998) and on the amount of available C (Simarmata et al., 1991).

The aim of the present study was to investigate the effect of N fertiliser forms, water supplies and decomposable organic matter on the gaseous N production in the soil air in model pot experiments.

Materials and methods

The two-year (1997–1998) model experiment was done with maize (hybrid Stira) as test plant under greenhouse conditions using large pots (diameter: 40 cm, depth: 40 cm). The pots were filled with 40 kg dry clay lessivated brown forest soil (clay = 24%, humus = 1.7%, $\text{pH}_{\text{KCl}} = 7.35$, USDA taxonomy: Eutrochrept). In both years, 150 mg N kg^{-1} soil was applied in the form of KNO_3 or NH_4Cl besides PK basic fertilisation before sowing. The experiment was done at two soil moisture levels, 65 and 100% of the maximum water-holding capacity (WHC) of the soil, with or without the incorporation of maize crop residues (R) into the soil (50 g dry powdered R per pot) and with or without maize test plants in 4 replicates. Watering and weekly weighing were performed to keep the above WHC levels. Gas-collecting traps of 1.8 dm³ volume with a sealed silicon pipe outlet were placed in the pot soil at a depth of 20 cm.

In both years ten plants per pot were grown until full maturity. Six and seven gas samplings were done during the growing season in the first and second year, respectively. The first samplings were done on the 14th day after fertilisation and the experiments were continued for 112 and 133 days in the first and second year, respectively. Soil air (5 cm³) was sampled with the help of hypodermic needles in three replicates per pot. On each occasion, the air of the experimental area was also sampled and used as a blank sample. The samples were analysed for NO, NO₂, N₂O and N₂ concentrations on a Carlo Erba gas chromatograph equipped with NP specific and thermal conductivity detectors and a Spectra-Physics SP 4000 data processing system. Nitrogen contents were calculated for the various gaseous N species from the gas analytical data, then these N amounts (NO-N, NO₂-N, etc.) were expressed in mg per total trap volume. Cumulative values were calculated as the sum of the amounts measured at the different samplings. Corrected values were obtained by subtracting the values measured for the control pots from those of the fertilised pots. These corrected values were used to express the total amounts of gaseous N evolved as the result of the different treatments as a percentage of the fertiliser N applied.

Results and discussion

Each fertiliser treatment resulted in significantly higher nitrogenous gas production at each sampling compared to the unfertilised controls. Both the total gaseous N amounts and the N amounts detected in the form of various N species showed a considerable increase during the vegetation period in all the treatments. (Examples are shown in Tables 1–2 and Figure 1). The intensification of nitrogenous gas evolution can be explained by the increase in soil microbial activity with rising temperature (Fillery, 1983; Mahmood et al., 1998). In the planted pots, the transformation processes of fertiliser N reached a steady-state condition on the 98th and 101st day after fertiliser application in the first and second experimental year, respectively (Figure 1). In the unplanted pots, the transformation processes continued up to the last sampling.

Table 1

Nitrous oxide amounts detected in gas traps in planted pots at different sampling times in 1997
(mg N₂O 1.8 dm⁻³)

Treatments	WHC	Sampling time (days after fertiliser application)					
		14	28	42	70	98	112
Control	100%	1.6	10.0	21.7	26.0	26.5	25.0
KNO ₃		34.3	50.5	94.0	129.0	144.5	158.0
NH ₄ Cl		28.1	53.7	95.5	147.3	147.7	135.6
Control	65%	1.5	8.8	20.5	23.3	24.6	24.1
KNO ₃		34.0	49.5	86.0	119.2	145.4	156.0
NH ₄ Cl		29.5	50.5	91.3	142.5	145.3	135.0
Control+R	100%	1.7	11.8	27.2	28.3	31.5	27.5
KNO ₃ +R		37.7	54.1	112.0	129.2	159.2	158.6
NH ₄ Cl+R		30.5	53.2	105.0	157.1	154.0	148.5
Control+R	65%	1.3	10.6	23.2	24.4	30.0	24.5
KNO ₃ +R		32.2	43.0	106.7	131.5	157.0	157.5
NH ₄ Cl+R		29.0	50.9	98.0	153.3	146.8	135.8
LSD _{5%}		2.4	3.0	4.4	4.0	4.4	6.0

Table 2

Nitrogen gas amounts detected in gas traps in planted pots at different sampling times in 1998
(mg N₂ 1.8 dm⁻³)

Treatments	WHC	Sampling time (days after fertiliser application)						
		14	28	42	56	70	101	113
Control	100%	70	90	111	137	149	149	150
KNO ₃		189	496	595	748	961	1183	1208
NH ₄ Cl		173	573	540	838	970	1171	1191
Control	65%	68	95	112	131	147	144	145
KNO ₃		185	495	565	760	1025	1138	1191
NH ₄ Cl		173	512	533	810	1050	1192	1185
Control+R	100%	70	97	121	141	157	150	150
KNO ₃ +R		188	550	550	813	992	1175	1192
NH ₄ Cl+R		180	580	598	869	1068	1188	1175
Control+R	65%	67	98	122	144	156	150	151
KNO ₃ +R		175	485	620	763	1060	1183	1179
NH ₄ Cl+R		174	558	600	876	1070	1205	1191
LSD _{5%}		4	24	33	17	30	28	21

The cumulative amounts of the different N-containing gases were substantially higher in the unplanted pots than in the planted ones. In both years, the incorporation of crop residues into the pot soil significantly increased the amount of all measured gas species in most cases (Table 3). Compared to the 100% WHC treatments, lower water supplies resulted in definite differences both in the planted and in the unplanted pots in 1997. In this year, a lower water supply significantly decreased the cumulative amount of all measured nitrogenous gases in each case. These results are in accordance with data from the literature on the stimulating effect of readily decomposable organic matter and increasing soil moisture on denitrification (Leick et al., 2001; Mahmood et al., 1998; Simarmata et al., 1991).

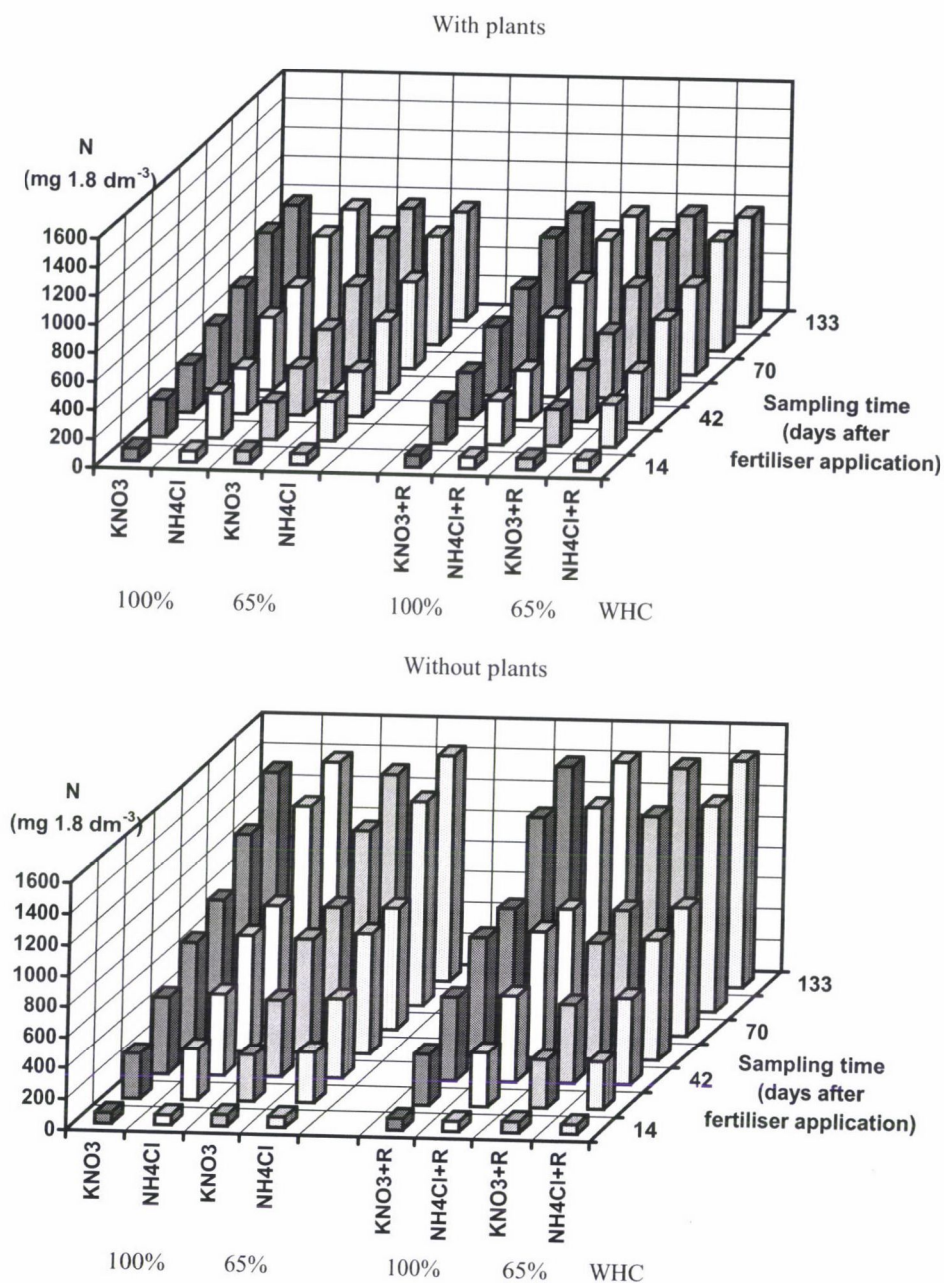


Fig. 1. Corrected values of total N contents of nitrogenous gases detected in gas traps at different sampling times (1998)

Table 3
Cumulative amounts of nitrogenous gases (mg 1.8 dm⁻³)

Treatments	WHC	1997				1998			
		NO	NO ₂	N ₂ O	N ₂	NO	NO ₂	N ₂ O	N ₂
With plants									
Control	100%	11	466	111	764	8	513	125	719
KNO ₃		174	1950	610	4677	193	2175	704	4632
NH ₄ Cl		179	1967	608	4730	198	2253	695	4618
Control	65%	8	439	103	718	8	523	122	711
KNO ₃		163	1891	590	4415	190	2149	714	4599
NH ₄ Cl		169	1931	594	4509	199	2246	731	4645
Control+R	100%	15	510	128	813	13	571	134	745
KNO ₃ +R		191	2110	651	4828	218	2302	767	4647
NH ₄ Cl+R		198	2071	648	4939	216	2427	779	4789
Control+R	65%	13	478	114	798	14	540	133	744
KNO ₃ +R		177	2041	628	4716	207	2286	766	4702
NH ₄ Cl+R		184	2034	614	4734	206	2413	769	4798
LSD _{5%}		2	12	4	22	2	12	3	20
Without plants									
Control	100%	13	370	87	489	13	428	100	479
KNO ₃		233	2270	867	7386	245	2738	1037	6359
NH ₄ Cl		206	2317	875	7305	271	2860	1049	6921
Control	65%	11	365	85	496	14	422	102	466
KNO ₃		224	2218	860	7137	264	2777	1040	6442
NH ₄ Cl		197	2279	863	7173	265	2837	1066	7092
Control+R	100%	16	385	98	510	17	449	114	492
KNO ₃ +R		256	2474	893	7696	279	2982	1071	6911
NH ₄ Cl+R		231	2516	927	7696	283	3023	1127	7117
Control+R	65%	14	386	93	502	15	440	112	473
KNO ₃ +R		244	2338	863	7454	277	2916	1061	6899
NH ₄ Cl+R		215	2399	887	7390	277	3004	1103	7163
LSD _{5%}		2	12	6	36	2	9	5	24

Compared to 65% WHC, the higher soil moisture level resulted in higher cumulative N₂O amounts in most cases (Table 3). This is in accordance with the results of Russow et al. (2000) and Wolf and Russow (2000), who found that increasing water saturation promoted the emission of the greenhouse gas N₂O.

The total amounts of gaseous N evolved, expressed as a percentage of the fertiliser N applied (Fig. 2), were similar in the experimental years and did not vary considerably with the different fertiliser treatments. Practically the same amounts could be measured with both N fertiliser forms: 12.6–13.1% and 23.1–24.6% in the different fertiliser treatments for planted and unplanted pots, respectively.

The composition of the nitrogenous gases evolved in the gas traps showed considerable changes during the vegetation period (Figure 3 illustrates the results for the first experimental year). The greatest differences in the gas composition could be observed between the unfertilised and fertilised pots.

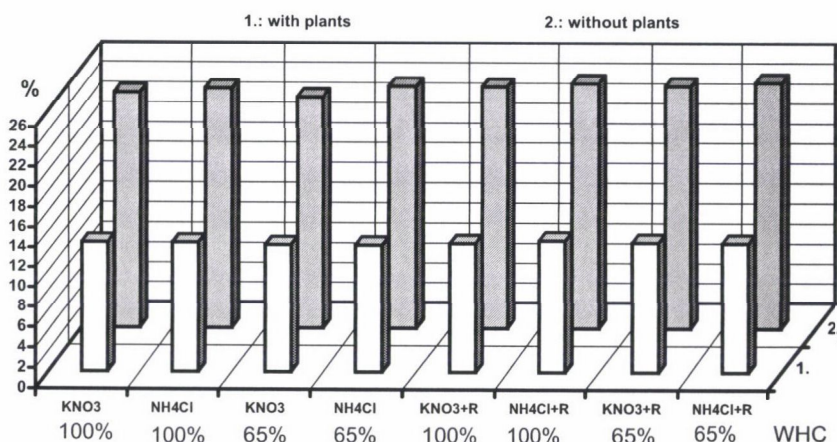


Fig. 2. Total amounts of gaseous N evolved as a percentage of fertiliser N, averaged over the experimental years.

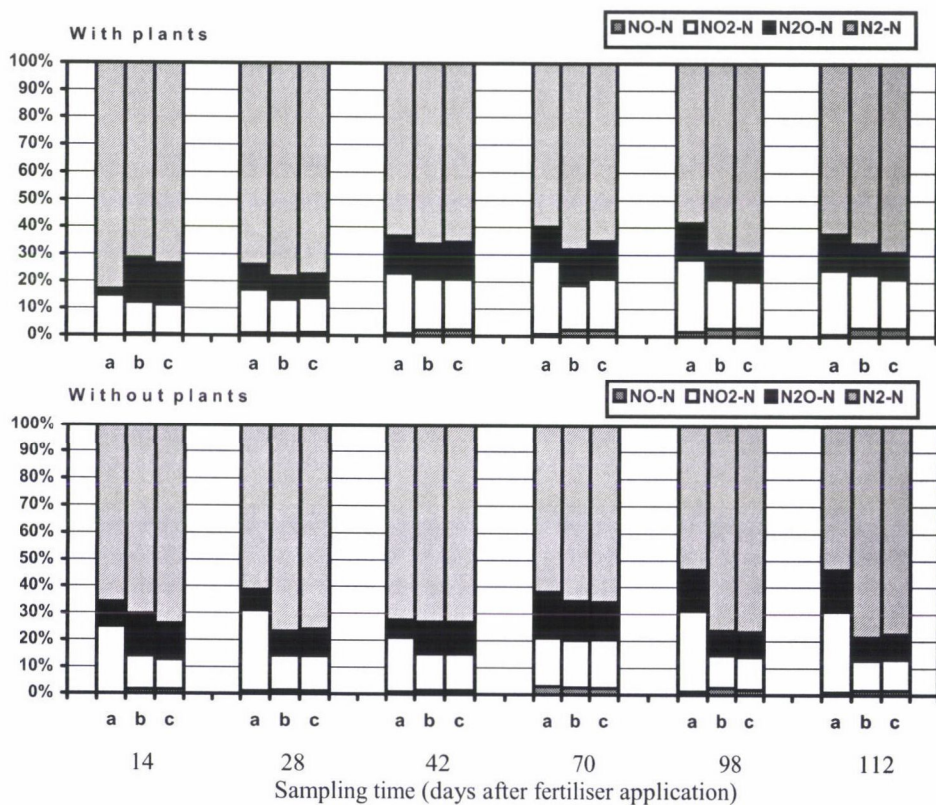


Fig. 3. Distribution of the different N forms within the total gaseous N detected in the soil air, averaged over WHC and R treatments (1997). (Fertiliser treatments: a = control, b = KNO₃, c = NH₄Cl)

Planted and unplanted treatments also showed considerable differences. The distribution of the different N-containing gases was very similar in the various fertiliser treatments. The ratio of the major gaseous end-product of denitrification, elementary N, was the highest in all the treatments, amounting to 77% in the fertilised unplanted treatments in both years. The rate of NO-N was the lowest, while NO₂-N and N₂O-N took second and third places in the order of N concentrations, respectively. On the two-year average, the ratio of the greenhouse gas N₂O-N amounted to 11.1 and 10.3% in the planted treatments and to 9.3 and 9.1% in the unplanted after KNO₃ and NH₄Cl fertilisation, respectively.

Conclusions

Similar gaseous N amounts were measured in the soil air with both N fertiliser forms. From the environmental point of view, water-saturated conditions and the incorporation of crop residues into the pot soil resulted in higher NO_x-N and N₂O-N ratios within the total gaseous N evolved in the rooting zone.

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Short communication

INVESTIGATION ON LOW DOSES OF ATRAZINE, METRIBUZIN AND PENDIMETHALIN ON WEEDS AND YIELD OF WHEAT

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The results obtained showed that there was severe competition between wheat and the predominant weed species *Phalaris minor*, *Avena ludoviciana* and *Melilotus indica*. Competition by other weed species was normal. The maximum reduction in grain and straw yields due to weed competition was 34.8% and 43.7%, respectively. Weed control treatments lowered the weed population and weed biomass accumulation and boosted up the crop yield. A significant reduction in the weed population and weed dry weight was observed with increasing doses of both atrazine and metribuzin. Metribuzin at 200 g ha⁻¹ killed all the plants of *M. indica* and gave excellent control of both *P. minor* (98%) and *A. ludoviciana* (89%). Atrazine at higher doses had an almost similar effect on *P. minor*, *A. ludoviciana* and *M. indica* with 83–87% control. Pendimethalin gave good control of *P. minor* and *M. indica* (75–83%) but poor control of *A. ludoviciana* (55%).

The highest yield was recorded in hand weeding which was significantly superior to all other treatments. Metribuzin at 100 g ha⁻¹ was the next best treatment, and this rate was superior to the higher doses. Pendimethalin and atrazine also brought about a marked increase in the crop yield. Higher doses of atrazine and metribuzin had a phytotoxic effect on the crop, reducing the number of productive tillers and finally lowering the crop yield, despite their excellent control of dominant weed species.

Key words: *Triticum aestivum*, herbicides, pendimethalin, atrazine, metribuzin

Introduction

In India wheat (*Triticum aestivum* L. emend Fioria and Poal) is the most important winter cereal crop and is the backbone of food security. It alone contributes a little more than one third of the total food grain production. Of late its productivity is declining. A formidable factor that limits its productivity is intense weed competition. Among the weed species *P. minor* poses a serious problem for wheat cultivation and on average it lowers crop yields by 30% (Pandey and Singh, 1997). With increasing weed population crop yields gradually decline. *P. minor* has developed resistance to the widely used potent herbicide isoproturon. Other herbicides used are narrow in selectivity. Thus it is imperative to find other powerful killers to eliminate weed competition and to ensure better crop growth. Atrazine and metribuzin at lower doses have been found most effective against *P. minor* as well as against other common weed species which invade wheat crop (Singh and Pandey, 1999). However, very little information is available on the efficacy of low doses of these herbicides in wheat. Hence a study was undertaken to discover the optimum dose of atrazine and metribuzin and their effect on weeds and on the yield of wheat.

Materials and methods

Site and soil

An experiment was conducted at the Indian Agricultural Research Institute, New Delhi (28° 38' N latitude, 77° 11' E longitude) during the winter season (Dec. to May of 1999–2000 and 2000–2001). The soil of the experimental plot was sandy loam with pH 8.0 to 8.4, organic C 0.54 to 0.65%, available P and K 11.0 and 180 kg ha⁻¹ in 1999 and 12.0 and 200 kg ha⁻¹ in 2000, respectively.

Experimental design and treatments

The experiment comprised nine treatments (Table 1) and was conducted in a randomized block design with three replications. The crop was fertilized with 100 kg N as urea, 18 kg P as single superphosphate and 35 kg K as muriate of potash. Half the dose of nitrogen and the full dose of P and K were applied at sowing, while the remaining N was applied at the first irrigation, given 25 days after sowing. The crop variety HD 2285 was sown 23 cm apart in lines with a tractor-drawn seed drill on December 3, both in 1999 and 2000, and was harvested in the first week of May in both years.

Field techniques

Pendimethalin [N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzeneamine] at 1 kg ha⁻¹ one day after sowing and atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] and metribuzin [4-amino-6(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one] at 100, 150 and 200 g ha⁻¹ 35 days after sowing were applied with the help of a knapsack sprayer fitted with a flat fan nozzle using a volume of 600 litres of water/ha. Hand weeding was done 25 and 45 days after sowing.

Table 1
Effect of treatments on weeds (70 days after sowing, pooled mean)

Treatments	Dose (a.i. g ha ⁻¹)	Weed population (No. 0.25 m ⁻²)	Weed dry wt. (g 0.25 m ⁻²)	Decrease in weed species (%)		
				<i>P. minor</i>	<i>A. ludoviciana</i>	<i>M. indica</i>
Weedy check	—	5.01 (102)*	4.74 (838) [†]	—	—	—
Hand weeding	—	2.16 (19)	1.16 (53)	98	100	87
Pendimethalin ⁺	1000	2.79 (31)	1.59 (101)	83	65	75
Atrazine ⁺⁺	100	2.89 (34)	2.74 (300)	73	50	70
Atrazine ⁺⁺	150	2.34 (20)	1.99 (158)	83	83	88
Atrazine ⁺⁺	200	2.19 (15)	1.49 (088)	86	83	82
Metribuzin ⁺⁺	100	2.62 (27)	2.53 (256)	88	88	84
Metribuzin ⁺⁺	150	2.36 (22)	2.17 (188)	95	88	92
Metribuzin ⁺⁺	200	1.81 (13)	1.52 (92)	98	89	100
S.Em ±	—	0.08	0.098	—	—	—
C.D. at 5%	—	0.25	0.307	—	—	—

P = *Phalaris*, A = *Avena*, M = *Melilotus*, ⁺ = pre-emergence, ⁺⁺ = post-emergence; *Figures in parenthesis indicate weed population per square metre and weed dry weight in kg ha⁻¹

Results and discussion

Effects of treatments on weeds

Phalaris minor (55 plants m⁻²), *Avena ludoviciana* (15 m⁻²) and *Melilotus indica* (30 m⁻²) were the dominant weed species which severely competed with the wheat crop. Besides these, *Chenopodium murale*, *Anagalis arvensis*, *Rumex* sp., *Fumaria parviflora*, *Convolvulus arvensis*, *Cyperus rotundus* and *Cirsium arvense* also competed, but the competition by these species was insignificant as their populations were very small. The competition effect was markedly reduced by the weed control treatments, as is evident from the significant decrease in the weed population and dry matter accumulation (Table 1). A significant reduction in the weed population and dry weight was observed with increasing doses of both atrazine and metribuzin. Singh et al. (1999a) also observed a marked reduction in the weed population and dry weight at higher doses of atrazine and metribuzin. Metribuzin at 200 g ha⁻¹ killed all the plants of *M. indica* and gave excellent control of both *P. minor* and *A. ludoviciana*. The growth of *P. minor* plants ceased completely a week after spraying with metribuzin; the plants became thin, leaves turned yellow, drooped and gradually dried. Atrazine at 100 g ha⁻¹ was comparatively less effective in controlling these three weed species, particularly *A. ludoviciana*, but at higher doses (150 and 200 g ha⁻¹) it had a similar effect to that of metribuzin, giving 83–97% control of all three species. Pendimethalin application resulted in a significant reduction in the weed population and dry weight as compared to the weedy check. It gave good control of *P. minor* and *M. indica* (75–83%), but poor control of *A. ludoviciana*.

Crop growth and yield attributes

Phytotoxicity due to the application of atrazine and metribuzin at 150 and 200 g ha⁻¹ was observed in both the years. The crop turned pale yellow, growth ceased and the plants remained stunted for 20 to 30 days. There was no marked reduction in plant height but a significant reduction in tiller production was recorded. Although the crop recovered from the initial adverse effect after a month, there was no improvement in tiller production.

Plant height and the number of productive tillers were markedly increased due to the weed control treatments. Hand weeding effected a significantly higher increase compared to all other treatments (Table 2). The effect of pendimethalin, atrazine and metribuzin was similar on plant height and the number of productive tillers, except metribuzin at 100 g ha⁻¹ which resulted in a significantly higher number of tillers compared to the other herbicide treatments. Higher doses of both atrazine and metribuzin (150 and 200 g ha⁻¹) gave excellent weed control but had an adverse effect on plant growth and the number of productive tillers.

Hand weeding resulted in the highest weight of grains ear⁻¹, number of grains ear⁻¹ and 1000 grain weight, which were statistically superior to other

treatments. The increase may be attributed to better crop growth owing to decreased weed competition during critical growth stages. Among the herbicide treatments, metribuzin at 100 g ha⁻¹ improved the yield attributes significantly, followed by pendimethalin and metribuzin at 150 g ha⁻¹. Atrazine was least effective, among the three herbicides, in improving yield attributes. All the yield attributes were adversely affected at higher doses of atrazine and metribuzin. This may be attributed to their adverse effect on crop growth.

Table 2
Effect of treatments on growth, yield attributes and yield of wheat (Pooled mean)

Treatments	Dose (a.i. g ha ⁻¹)	Plant height (cm)	Maximum tillers m ⁻¹	Wt. of grains ear ⁻¹ (g)	No. of grains ear ⁻¹	1000 grain weigh (g)	Grain yield (kg ha ⁻¹)	Straw yield (kg ha ⁻¹)	Harvest index (%)
Weedy check	—	81.5	87.0	1.52	36.63	35.91	3003	5152	36.47
Hand weeding	—	100.0	129.0	2.36	54.75	43.56	4609	9154	33.57
Pendimethalin	1000	88.5	104.5	2.03	48.52	40.73	4270	7354	36.78
Atrazine	100	88.0	103.5	1.97	46.42	39.98	4106	6626	38.19
Atrazine	150	89.0	102.5	1.95	46.33	39.23	4414	6700	37.55
Atrazine	200	87.8	102.0	1.92	46.08	39.50	4030	6743	37.88
Metribuzin	100	89.6	108.5	2.16	49.75	41.46	4380	7325	37.53
Metribuzin	150	89.0	103.5	2.08	47.86	40.31	4054	6738	37.65
Metribuzin	200	88.5	101.0	1.94	44.75	40.25	3973	6332	38.73
S.Em±	—	0.65	1.03	0.01	0.33	0.09	17.00	129.0	0.41
CD at 5%	—	2.04	3.20	0.04	1.02	0.27	49.00	401.0	1.27

Grain and straw yield

Weed competition lowered the grain and straw yields by 34.8% and 43.7%, respectively. Hand weeding gave excellent control of weeds and resulted in the highest grain and straw yields, which were significantly superior to other treatments (Table 2). Metribuzin at 100 g ha⁻¹ was the next best treatment. It proved significantly superior to all its other doses, to atrazine at all the doses and to pendimethalin. The higher increase may be ascribed to the excellent control of weeds, and the marked increase in crop growth, productive tillers and yield attributes. Similar observations were made by Singh et al. (1999b). Grain yield was significantly higher after treatment with pendimethalin as compared to atrazine. Pendimethalin at 1000 g ha⁻¹ and metribuzin at 100 g ha⁻¹ resulted in significantly higher straw yield as compared to atrazine (100–200 g ha⁻¹) and to higher doses of metribuzin. Higher doses of both atrazine and metribuzin (150 and 200 g ha⁻¹) also had an adverse effect on grain and straw yields. This finding is in contrast to the findings of Singh et al. (1999a), who recorded an increase in grain and straw yield with increasing doses of atrazine (150g ha⁻¹) and metribuzin (200g ha⁻¹). The lower yield may have been due to the detrimental effect on crop growth, productive tillers and yield attributes, despite excellent control of the dominant weed species. Different doses of atrazine and metribuzin

had a similar effect on the harvest index, which was significantly superior to the rest of the treatments. The harvest index was similar for pendimethalin and the weedy check. A significantly lower harvest index was recorded in hand-weeded plots, which may be attributed to the profuse crop growth and marked increase in tillering owing to the better crop growth environment ensured by the elimination of weed competition during the growth period.

The present study suggests that to achieve higher crop yields, the crop must be kept free of weed competition from the early crop growth stages. Atrazine and metribuzin at lower doses and pendimethalin brought about a marked increase in yield attributes and crop yield owing to the effective control of grassy and broad-leaved weeds.

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Review

SIGNIFICANCE OF THE EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* HÜBN.) IN MAIZE PRODUCTION

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The European corn borer (*Ostrinia nubilalis* Hübn.), which is to be found almost universally in Europe and America, is an extremely important pest from the economic point of view. Losses caused by the pest range from 250–1000 kg/ha depending on the degree of infestation, the year and the yield averages. This fact justifies protection measures in Hungary on the whole of the seed production and sweetcorn fields and on 40% of the commercial maize sowing area. In addition to the direct damage, indirect losses are also considerable, since the injuries caused by the pest facilitate infection by *Fusarium* species. For the above reasons it is worth reviewing the habits of this pest, the extent of the economic loss resulting from the damage, and ways of controlling it.

Key words: European corn borer (*Ostrinia nubilalis* Hübn.), pest habits, pest damage, control measures, resistance breeding

Introduction

The European corn borer (*Ostrinia nubilalis* Hübn.) belongs to the Pyraustidae family. It is of Eurasian origin, and is now found throughout Europe with the exception of Scandinavia and the British Isles, but the greatest damage is caused in Southern and South-Eastern Europe. It was also imported into North America, probably between 1906 and 1916, and later appeared in the Far East, the eastern part of North Africa and in the West Indies. The most important zones where serious losses are suffered are the North American Corn Belt, the foothills of the Caucasians, and maize-growing areas in the Trans-Caucasian areas of South-Eastern Europe, where damage is caused by several generations a year. In the Carpathian Basin the pest is generally widespread in areas located 500–700 m above sea level; major damage zones are in the southern part of the Basin (in the northern parts of Yugoslavia), while severe damage is also caused less frequently in the southern part of the Trans-Tisza region (the Hungarian Corn Belt), on low-lying areas of the Great Hungarian Plain alongside the River Danube, and in parts of Transylvania (Romania). Less severe damage is also incurred in Central and Western Hungary (Nagy, 1993).

Maize is the preferred host plant of the pest, despite the fact that maize is indigenous in America and the corn borer in Eurasia. It is interesting to note that it was never able to spread in such great proportions on its original hosts (hemp, hops, millet and sorghum) as it has in maize (Manninger, 1949).

Literary review

The European corn borer (*Ostrinia nubilalis* Hübn.)

Description of the pest

The imago has a wingspread of 20–30 mm and measures 14–16 mm in length. The sexes differ considerably (in 1796 they were described as two different species). The forewings of the male are violet-brown and the back wings grey-brown, with yellowish white stripes and patches. The female has yellow wings, with thin brownish zigzag stripes. The female lays clusters of 25–50 eggs (Balázs and Mészáros, 1998).

The egg clusters are yellowish white or ivory-coloured, round or oval in shape, and have a diameter of 4–10 mm. A day or two before hatching they begin to turn grey with dark points where the heads of the larvae show through. This is known as the black-headed stage. When the larvae hatch they are 3–4 mm in length and are greyish white with black heads. They pass through 5–7 larval stages, during which time their heads gradually turn light brown (Nagy, 1993). The fully developed larva is yellowish or greyish white, occasionally with a violet or pink flush; there are darker diffused stripes running along its back. It is naked, with very few bristles, and measures 25–30 mm in length. The chrysalis is 13–16 mm long, slim, and ochre yellow or brown in colour; it is surrounded by a loose, transparent cocoon (Nagy, 1968). The chrysalises differ morphologically, the females being larger and heavier (Nagy, 1993).

Development cycle

The development cycle of the European corn borer exhibits great variability in different regions, obviously due to the large range of ecological habitats it occupies. In Central Europe it has one generation a year; the boundary of the two-generation area runs from Southern France and Italy across the Southern Balkans towards the Caucasus.

In Hungary the development cycle of the European corn borer is as follows: after overwintering, the fully developed larvae pupate in May or June and the first (spring, early summer) generation of moths emerge from the end of May to the middle of July. The eggs laid during this period hatch after 5–10 days and the larvae feed for 4–6 weeks until they are fully developed. Some of the larvae pupate in late July or early August and are transformed into moths without a diapause. The moths belonging to this second (summer) generation contribute little or nothing to the maintenance of the moth population. This type of development cycle is characteristic of the southern and south-eastern part of Hungary, but has also been observed elsewhere (Hanó, 1976; Pálffy, 1976; Gyurkó, 1980). The rest of the larval population, the size of which varies from year to year, overwinters and it is these individuals which ensure the continuity of the Hungarian population. The diapause, which lasts from August to April or May, is thus an extremely important part of the development cycle (Nagy, 1993). The beginning of the diapause is indicated by delayed gonad development, the cessation of feed uptake, the lack of pupation and a reduction in oxygen consumption (Beck and Hanec, 1960).

Habits of the imago

The imagos are active at dusk and during the night (between 8 p.m. and 8 a.m.) (Manninger, 1949). If disturbed during the day the moths fly 5–20 m and then resettle in the depths of the vegetation, but they can fly well and may cover several kilometres during the night (Nagy, 1993).

Their sexual activity is regulated by the sex pheromone production of the females. This compound, which attracts the males, was isolated from the females and then synthetically produced by Klun (1968) and Klun and Brindley (1970), who identified it as 11-tetradecenyl-acetate (11-tda). Males of populations from various geographical regions are attracted by different ratios of the two (cis and trans) isomers. Mating activity is induced by the reduction in temperature and light intensity at dusk (Sparks, 1963, cit. Nagy, 1993; Loughner and Brindley, 1971, cit. Nagy, 1993) and reaches a peak around midnight. High humidity and moisture not only enhance the sexual activity of the males (de Rozari et al., 1977), but also improve the fertility of the females. In hot, dry weather not only does their fertility decline, but the eggs fail to adhere to the plant properly and 85–90% may fall to the ground (Radulescu et al., 1960). In years when the fields were regularly waterlogged (during the periods 1940–1945 and 1952–1957) Manninger (1975) registered severe infection with European corn borer (100% infestation was recorded in Fejér and Tolna counties in 1944).

Egg-laying continues for 2–3 weeks, but the majority of the eggs are laid within the first 4–10 days at around 23°C, while some 80% have been laid by the 11–13th day (Nagy, 1973). The choice of the most suitable plant for oviposition is the task of the female. Everly et al. (1979) found that the females were attracted by well-developed, tall maize plants. The eggs are deposited in clusters of 16–18 on the better developed ears or on the back of the upper leaves, close to the midrib. The females lay an average of 250–300 eggs, though in exceptional cases they may produce twice this number (Huzián, 1982). They try to choose plants which will ensure both the feeding and overwintering of the larvae, so they generally deposit their eggs on tall, well-developed plants which will mature by the autumn (Manninger, 1949).

Guthrie et al. (1983) examined the correlations between corn borer infection and plant height and found that there were fewer larvae on shorter plants, a fact they explained by the physiology of the plants. According to Williams and Davis (1984), in susceptible hybrids an increase in the infestation level led to a reduction in plant and ear height, while resistant hybrids did not respond to the infection. Russell et al. (1979) established the fact that the difference between resistant and susceptible plants could be attributed to changes in allele frequencies for plant height and ear height.

Larval development and habits

Towards the end of embryo development the head gradually becomes darker. A few hours after this stage is reached the L₁ larvae hatch (Nagy, 1993). Dull, cloudy weather with a temperature of 20–22°C and 70–80% humidity is required for embryonic development and hatching (Manninger, 1949). All the larvae hatch from the egg clusters at the same time, even if it contains as many as 30–40 eggs. If hatching is protracted, it is a sign that the moths had poor vitality or were infected (Dulizibaric, 1966, cit. Nagy, 1993). After hatching the larvae exhibit great vagility, crawling all along the edges of the leaves and possibly passing to another maize plant by letting themselves down on a silk thread. The migrating instinct of the L₁ larvae ceases after the first day and they become negatively phototropic, settling in the protected leaf axils and beginning to feed. From then on the larvae lead a concealed existence. The first three developmental stages are spent in the protected region between the husk and the stalk. Later the larvae penetrate into the stalk and bore tunnels in the pith, moving gradually downwards towards the lower, thicker part of the stalk. In some cases, especially when there is a large larval population, the corn borers may penetrate the ears (Nagy, 1993). According to Bakó (1926) some of the larvae penetrate the ear immediately, while others enter it from the stalk. Caffrey and Worthley (1927) found that as the larvae developed they were able to go for ever longer periods without eating: this was 1.95 days for the L₁ larvae and 22.8 days for the L₅ larvae. Benedek and Hanó (1986) demonstrated that mortality was high during egg hatching, amounting to 72.6% in their experiments. There were several reasons for this: there is often a long period of dry weather during the egg hatching period, young maize plants contain toxins poisonous to the larvae, and for the first few days after hatching the larvae are found on the plant surface, where they may be subject to mechanical injury. Another critical period is overwintering, since around 90% of the larvae may be destroyed by soil cultivation in autumn.

Overwintering, pupation

The climate of the Carpathian Basin forces the corn borer to overwinter. Fully developed larvae are capable of withstanding temperatures as low as –20°C for three months or more, provided they are not in contact with moisture (Hanec and Beck, 1960). This subject was studied in Hungary by Pletser (1962) and Dolinka and Pletser (1962).

The larva builds its chrysalis in the feeding cavity in the maize stalk, first forming the hole through which it will emerge and covering this with a protective web (Nagy, 1993). However, if forced to leave the maize stalks the larvae are also capable of overwintering in the stems of herbaceous weeds (Virágh, 1956).

Within a few hours the originally light-coloured chrysalis turns brown. The course of pupation differs for diapausing and non-diapausing larvae. Those which pupate in summer without a diapause generally find enough moisture in the green stalks, while the overwintering larvae can only begin to pupate in the dry stalks if there is sufficient rainfall. This generally occurs in April or May, but if the spring is dry it may be months before they are able to pupate. In the meantime they suffer gradual water loss and many of them die. Withholding the moisture required for pupation was thus suggested at one time as a means of pest control (Bakó and Jablonowski, 1926). At temperatures above 30°C the pupation period is around 7 days, but otherwise it is completely temperature dependent. Based on the daily temperature maxima summed from April 1st, Manninger (1949) calculated that the heat sum required for 70% pupation and for the beginning of moth emergence was 1495.2°C. The moths are able to fly within a few hours of emergence.

Hertelendy (1977) found that the quantity of rainfall and the value of Seijanihon's hydrothermic coefficient during the pupation period were in close negative correlation ($p = 0.1\%$) with the date of the flight peak. The more rain there was in May, the sooner the flight peak occurred. Hertelendy (1977) calculated the date of the flight peak using the linear equation $y = 812 - 2.44x$, and the values obtained were very close to the actual flight peak data. It thus appears that the quantity of rainfall during the pupation period has a great influence on the flight peak date.

Type and extent of damage

Freshly hatched larvae start to feed on the youngest maize leaves from the middle of June, leaving irregular holes in the leafblade which can be distinguished from damage caused by other pests (e.g. cotton bollworm) by the presence of finely chewed leaf debris attached to the leaf by webs. In drier regions these first signs of leaf feeding may not be visible, since the low air moisture may induce the larva to penetrate into the stalk at an early stage.

The following type of damage is observed when the larvae bore into the tassel stalk, often causing the tassel to break off. The site of entry is betrayed by a small pile of whitish, light-brown excrement resembling sawdust.

Older larvae may penetrate into the soft kernels on the ear, into the cob or into the cob shank, thus promoting the development of ear rot. If this coincides with a period of wet weather the likelihood of mould developing is even greater.

Leaf feeding causes a certain reduction in assimilation, while stalk feeding leads to a deterioration in the physiological status of the plant, depending on the extent of infestation. This in turn influences the quantity of yield. Penetration into the stalk also opens the way for fungal diseases (Nagy, 1993).

Szentirmay and Szolnok (1976) studied the relationship between corn borers and fusarium infection. In 1975, when the experiments were carried out, the weather was excellent at harvesting. It was a hot, dry year with little rainfall,

so there was virtually no fusarium infection as the result of wet weather. It was therefore possible to compare the two parameters realistically, and linear regression showed a positive correlation ($r = 0.7$).

Mile and Ilovai (1979) estimated that a 10% infection led to a yield loss of 0.5–0.6 t/ha, though in wet years the damage was even greater at the same level of infection.

Experiments carried out by Pálffy (1983) were aimed at determining the proportion of stalk lodging induced by corn borers and by fusarium. The results suggested that in the vast majority of cases (89.67%) lodging was caused by corn borers, and only 7.33% of lodging could be attributed to fusarium infection. In post-harvest studies it was noted that almost all the stalks which lodged due to corn borer damage were also infected with fusarium, and 22% also showed signs of ear fusarium.

Korom et al. (1985) examined the pathogens, host plants, vectors and environmental factors involved in the development of fusarium ear rot and found that the corn borer played a dominant role as a vector of fusarium ear rot in Southern Hungary in 1982–1984 ($r = 0.802$). Fusarium infection was observed to begin after tasselling on the adult plants.

According to Herczig et al. (1987) the two-generation populations in warmer parts of Hungary caused the greatest damage.

Szeőke et al. (1996) reported that injuries caused by moth pests played an important role in the development of fusarium infection, which increased greatly in moth-infested crops.

After two years characterised by severe outbreaks of fusarium ear rot, Csörnyei et al. (1997) elaborated a plant protection technology effective against ear rot infection, corn borers and corn earworm.

Szőke (2000) found fusarium infection as the result of corn borer damage in maize genotypes in the very early and early maturity groups. Hertelendy (1978) examined the relationship between the vegetation period of maize and infestation with corn borers in experiments carried out in Zala County and found that varieties with shorter vegetation periods suffered a greater extent of infestation.

When maize was artificially infested with 1 larva per plant, Patch et al. (1942) recorded yield losses of 3.5%, 2.85% and 1.9% when the larvae were placed on the plants on June 27th, July 8th and July 18th, respectively. In experiments involving 4323 maize plants, Manninger (1960) recorded yield losses of 20% at an infestation rate of 68%. Measurements made by Dolinka (1960) showed that early varieties suffered losses of 3.6–5.9%, mid-early varieties 2.6–2.9% and mid-late varieties 7.7%. This author considered the losses to be caused partly by biological damage to the vascular bundles of the leaf and stalk during feeding and partly to mechanical injury when the damaged stalks break off below the ear or lodge at the base of the stalk. Hertelendy and Szabó (1976) observed yield losses of 11% at an infestation rate of 68.2–69.7%.

According to Dolinka (1979) the losses arising due to disturbed nutrient transport amount to 0.5–3.6%, while the mechanical damage suffered by ears lying on the ground causes losses of 20–50%. Pálffy (1983) reported yield losses of up to 20% and divided these losses into three groups: primary losses arising from weight reduction, secondary losses due to the extra costs required to harvest ears on broken stalks, and additional losses, since the weight reduction depended on the number of larvae found in the stalks. This author found a correlation between corn borer infection and ear weight reduction which was significant at the 0.1% level, indicating that the difference in ear weight between healthy and infested plants could be attributed with 99.9% certainty to the corn borer.

Davis et al. (1978) demonstrated that the more eggs were laid on each plant, the shorter the plant would be as a result of larval feeding.

Papp (1990) found yield losses of as much as 15–20% due to direct (disturbed nutrient transport) and indirect (harvesting losses, attack by secondary pathogens) damage. Bohn et al. (1999) reported that in the case of early maize hybrids a 1% increase in the number of infested plants led to a 0.28% increase in the yield losses, while each larva/plant caused a yield loss of 6.05%.

Keszthelyi and Sáringer (2002) examined changes in chemical quality as the result of corn borer damage and found a positive correlation between the reduction in the crude fat content and the extent of damage. There was also a positive correlation between the reduction in the starch content and the extent of damage.

Basic principles of control

Agrotechnical control

The oldest measures used to control corn borers belong in this group, and many of them can still be used today extremely successfully, with no extra cost. A ministerial decree (No. 43/1968) states that maize stalks must be destroyed at the latest by May 15th of the following year. Bakó (1926) summarised the fundamental agrotechnical measures in his “ten commandments for corn borer control”. Jablonowski (1927) drew special attention to the proper treatment of maize stalks. In small farms, where the stalks are fed to animals, the larvae are able to overwinter in farm buildings, in the bark of trees or in dry manure, as proved by the fact that the maize fields closest to the villages were always the most infested. According to Nagy (1968) many larvae can be removed by detasselling around the flowering period (in the case of seed production), but care must be taken to ensure that these cannot return to the plants.

Biological control

Of the many natural enemies of the European corn borer, the most important are the egg parasite *Trichogramma* (90% infection with parasites was

recorded in the Nyírség region of Hungary) and the larva parasites *Lydella* and *Campoplex*. The corn borer is attacked by several pathogens, including the protozoon *Perezia pyraustae*, the fungi *Metarrhizium anisopliae* and *Beauveria bassiana* and the bacterium *Bacillus thuringiensis* (Nagy, 1968; 1993).

Siegescu (1928) reported that international projects had been set up to study the insect parasites and pathogenic microorganisms which are the natural enemies of the corn borer. Hataláné-Zsellér and Mile (1992) investigated bioherbicides containing *Bacillus thuringiensis varietas kurstaki* as active agent and recommended the application of BIOBIT WP at a dose of 1.5 kg/ha or BIOBIT XL at a dose of 2 l/ha in 50 l water/ha using ULV equipment to control corn borers. Ivezic and Raspudic (2001) were able to reduce the intensity of corn borer damage by 46% with bioherbicide (BIOLIT XL), while reductions of 98% and 21% were achieved with the help of two Bt hybrids. Hluchy and Szeőke (2002) tested a control technology based on *Trichogramma* egg parasites and recorded an average efficiency of over 80%. The egg parasites were placed on the sheaths of the maize plants in perforated plastic capsules (2000 parasites/capsule), first when the moths began to fly and on a further 2–3 occasions at 5–7-day intervals.

Chemical control

In Hungary the degree of infestation rarely reaches the level where chemical control is economic, so it is rarely applied except in sweetcorn and for seed production. Many papers have appeared in both foreign and Hungarian journals on the chemical control of corn borers, but these will not be detailed here, since the chemicals recommended continually change. The main principles of chemical control were defined by Nagy (1993) as:

- the use of a wide range of chemicals (phosphoric acid esters, thiophosphoric acid derivatives, dithiophosphoric acid and carbamate derivatives, piretroids, etc.)
- granulated compounds are recommended as they remain effective for a longer period and also cause less damage to useful entomophages
- treatment should coincide with the egg hatching peak
- treatment of large areas can best be achieved from the air
- the presence of possible chemical residues must be taken into consideration when feeding maize stalks to animals.

Resistance breeding

As mentioned above, chemical control of corn borers is rarely economically justified in Hungary. For this reason, the use of hybrids resistant or tolerant to corn borers and breeding for such resistance are of great importance. Resistance is based on the morphological and chemical properties of the plant, but the climate of the particular year and the stage of development of the plant may modify the genetically determined resistance. Morphological resistance breeding is based on the physical properties of the plant.

Corn borer larvae find it more difficult to penetrate maize plants with harder stalks, so there is less danger of tassel or stalk breakage (Nagy, 1993). A distinction must be made in resistance breeding between the first and second generations of the corn borer. Resistance to the first generation is primarily leaf feeding resistance and originates from the structure of the leaf tissue. Lines CI31A, OH43, A619 and B49 carry genes conferring this type of resistance (Guthrie et al., 1970; 1972). Resistance to the second generation is primarily sheath and collar feeding resistance. According to Dolinka (1977) maize resistant to both generations can be bred in two ways: 1. by crossing lines each resistant to one of the generations ($B52 \times OH43$, $B52 \times B49$, $B52 \times CI31A$), but these are all too late-maturing; 2. by developing composites also containing lines with good lodging resistance and yield potential. Guthrie et al. (1982) examined 99 hybrids developed from the most widely used, freely available inbred lines in experiments carried out in Iowa and Taiwan. In Iowa two lines were found exhibiting resistance to 2nd generation larvae (SC213 and B86), while on Taiwan these lines were also susceptible. Line B52 was resistant to 2nd generation larvae at both locations.

Rojanaridpiched et al. (1984) dealt with resistance based on chemical properties and found that resistance to the first and second generations of the corn borer was correlated with the 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3-(4H)-one (DIMBOA), SiO_2 and lignin content of the maize. However, the hybrids generally had different levels of resistance to the two generations of larvae, so breeding must be carried out separately for each.

Ordas et al. (1988) examined the resistance of 16 hybrids of different origin against 2nd generation larvae. Three early populations proved to be most resistant on the basis of number of stalk cavities, so it was assumed that early varieties were the best initial stock for resistance breeding.

Kim et al. (1989) investigated the genetic control of resistance to 2nd generation larvae in inbred maize lines. Significant differences were found between the resistance levels of the 9 parents and 36 hybrids, 82% of which was attributed to general combining ability and 18% to specific combining ability.

Guthrie and Jarvis (1990) raised corn borer larvae on the resistant lines CI31A and OH43 for 22 years in order to determine how long they retained their resistance, but no biotypes evolved which were capable of breaking down the resistance. Eight genes were found to code for resistance to leaf feeding.

Barry et al. (1994) studied resistance to the 1st generation of corn borers in 2 resistant and 2 susceptible inbred maize lines and in all possible F_1 and F_2 generations. The resistance levels of the F_1 and F_2 hybrids were generally determined by those of the parents. Resistant genotypes had a higher DIMBOA concentration, which exhibited additive inheritance in the various crosses, indicating that the DIMBOA concentration is genetically determined. As the leaves matured the DIMBOA concentration declined, while the larva damage increased.

Abel and Wilson (1999) investigated the action mechanism of resistance by studying the behaviour of corn borer larvae on a resistant genotype (CI31A) and on a maize variety from Peru. The weight of larvae fed on the leaves of the Peruvian maize did not differ significantly from that of larvae feeding on CI31A leaves, indicating that the extent of antibiosis in the Peruvian maize was similar to that in inbred line CI31A, which has a high DIMBOA concentration. After 5 days the number of larvae on the Peruvian maize decreased significantly, not to such a great extent as in the resistant genotype, but more than in the control (WF9). This suggests that non-preference may be the basis of resistance in the Peruvian maize. If Peruvian leaves were mixed with the normal (control) diet, the effect of the resistance factor disappeared. There could be several reasons for this: the components of the normal diet may have masked the resistance factor, or it could be that the resistance factor consists of the deficiency of a nutrient essential for larval development, which they obtained in the mixed diet.

Cardinal et al. (2001) searched for the loci of QTLs responsible for corn borer resistance. Nine QTLs found on the maize chromosomes were responsible for 59% of the genetic variance. Six QTLs leading to reduced stalk damage originated from the resistant parent, B52. One digenic interaction was found among the QTLs. In Hungary Dolinka (1960; 1978) and Vörös (1978) bred or discovered hybrids and lines resistant or tolerant to European corn borer.

Pásztor and Borsos (1990) crossed 14 different inbred maize lines with a teosinte male and observed that in the F_2 generation of the hybrid combinations resistance to fusarium and corn borer was exhibited. This was interesting because the plant height of both the F_1 and the F_2 generations exceeded that of the parents, and corn borer moths usually prefer to lay their eggs on taller plants. It is thus probable that the resistance is due to the chemical composition of the hybrids.

Gyulavári et al. (1999) obtained moth-resistant basic stock from America, which they selected for resistance to the Hungarian moth population. Resistant analogues of several valuable lines were developed using the backcross method. Hybrids were found which suffered no stalk breakage and produced high yields despite larval feeding in the stalks. This demonstrates the fact that attention should also be given to tolerant lines.

Biotechnological control

In the course of sporulation, *Bacillus thuringiensis* (Bt) produces white crystals with an insecticide effect, exhibiting specific toxicity to insects belonging to the Lepidoptera and Diptera. When the crystals dissolve in the digestive system of the larvae a protein with a molecular mass of 130–160 kD is released, from which a low molecular mass toxic fragment is split off by proteases. The gene has been cloned from a number of Bt subspecies (e.g. *kurstaki*, *berliner*), and the crystal and spore preparations have been successfully applied against insect pests. It was assumed that if the Bt toxin gene was expressed in the plant, this would make spraying with the toxin unnecessary (Dudits and Heszky, 1990).

Transgenic Bt plants were first registered in 1995 by the United States Environment Protection Agency (USEPA). When they were first cultivated in 1996 they made up only 1% of the total maize sown. This proportion had risen to 17% by 1998 and to 20–25% by 1999. So far three different Bt toxins have been introduced into commercial hybrids (Cry1Ab, Cry1Ac and Cry9c) (Edwards, 1999).

Barry et al. (1997) examined the resistance of 400 commercial maize hybrids to both generations of corn borer and found that 90% of the hybrids had some level of resistance to the 1st generation and 75% to the 2nd generation. It proved possible to intensify the moth resistance in two-thirds of the 400 hybrids. Maize genotypes transformed with the Bt gene efficiently resisted corn borer infestation in all developmental stages. Graeber et al. (1999) evaluated a transgenic maize hybrid containing a Bt gene and reported that the leaf and stalk damage caused by both generations of larvae was reduced or non-existent. Neither the grain yield, stalk lodging nor mass of the transgenic hybrids was influenced by either generation of the corn borer, while in control (non-transgenic) hybrids the grain yield decreased by 0.4–6.6% as the result of 2nd generation damage, there was a slight increase in lodging, and total plant mass was reduced. In the absence of corn borer infestation there was no difference in the yield of transgenic and traditional hybrids.

Clark et al. (2000) compared the efficiency of Bt maize hybrids and two traditional control methods against the European corn borer. The treatments included transgenic Bt maize hybrids, the non-transgenic isogenic lines of these hybrids, and the application of insecticide (permetrin) or bioherbicide containing Bt active agent to these lines. The following order was obtained for efficiency against corn borers: transgenic Bt maize > traditional insecticide (permetrin) > bioherbicide containing Bt active agent > control. Transgenic maize gave the greatest protection against ear damage and in general this type of maize produced the highest yield.

In a comparison between Bt hybrids, their non-transgenic variants and commercial hybrids, Magg et al. (2001) found that the Bt hybrids exhibited a significantly lower level of infestation than the non-transgenic variants and the commercial hybrids.

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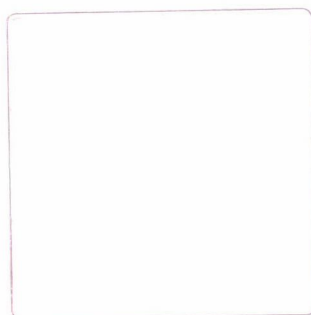
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Review

TETRAPLOID WHEAT SPECIES *TRITICUM TIMOPHEEVII* AND *TRITICUM MILITINAE* IN COMMON WHEAT IMPROVEMENT

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Timopheevii wheats are discussed as donors for improving the disease resistance of common wheat. Attention is paid to the comparison of the morphological and chromosomal characteristics of *Triticum timopheevii* and *T. militinae*, their crossability with *T. aestivum* and their response to fungal diseases. The possible origin of *T. militinae* from an introgressive hybridization between *T. timopheevii* and an unknown species is discussed. Major genes for resistance to various fungal diseases, transferred to common wheat from *T. timopheevii*, are listed.

Key words: *Triticum timopheevii*, *T. militinae*, disease resistance, wide hybridization

Introduction

In addition to the emmer group, with the genome formula AABB, the genus *Triticum* also includes tetraploid wheats of the timopheevii group (*Triticum timopheevii* Zhuk.), with the genome formula A¹A¹GG. This group includes the wild form of timopheevii wheat known as *Triticum timopheevii* (Zhuk.) Zhuk. var. *araraticum* (Jakubz.) (further abbreviated as *T. araraticum*), the domesticated form *Triticum timopheevii* (Zhuk.) Zhuk. var. *timopheevii* (further abbreviated as *T. timopheevii*) and also a third form of wheat discovered by P. M. Zhukovsky in 1950, *Triticum militinae* Zhuk. et Migusch. (further abbreviated as *T. militinae*).

In the present review, the possibilities of using timopheevii wheats as donors for improving the disease resistance of common wheat will be discussed. The main attention will be paid to the comparison of the characteristics of *T. timopheevii* and *T. militinae*.

Origin and description

The wild form of timopheevii wheat, *Triticum timopheevii* (Zhuk.) Zhuk. var. *araraticum* (Jakubz.), is found throughout the Middle East and surrounding areas, including Transcaucasia. Plants of *T. araraticum* are characterized by a high content of grain protein (up to 30%), resistance to powdery mildew (caused by *Blumeria graminis* (DC.) E. O. Speer f. sp. *tritici* Em. Marchal [syn. *Erysiphe*

graminis f. sp. *tritici*]) and smut (caused by *Ustilago tritici* (Pers.) Rostrup). However, according to Dorofeev (1976), poor yields, hulled brittle ears and susceptibility to stripe and stem rusts make it difficult to use this species in wheat breeding. Wide intraspecific diversity, accompanied by chromosomal aberrations, mainly translocations, has been found in this form of the species (Kawahara and Tanaka, 1981; Badaeva et al., 1994a; 1995a).

Wild timopheevii wheat is considered to be the progenitor of *Triticum timopheevii* (Zhuk.) Zhuk. var. *timopheevii* (Feldman, 1977), a domesticated form which was cultivated as a population mixed with *T. monococcum* (Chelata-Zanduri population) in western Georgia, where it was discovered by P. M. Zhukovsky in 1922. Zhukovsky (1928) described the form as follows: The plants have thick pubescence, characteristic of the species, consisting of long white tough hairs on the leaf-sheaths, with thinner pubescence on the leaf-blades. The ears are compact (40–45 spikelets/10 cm of spike rachis; Dorofeev, 1976), broad, pyramidal and flattened, with the width considerably exceeding the thickness. Only forms with pubescent ears are known. The ears are brittle at maturity; segments of the rachis are pubescent with comparatively thin short hairs. The awns are soft, thin and 4–7 cm in length. The glumes are thin and membranous. The empty glumes are much shorter than the lower flowering ones (Zhukovsky, 1928).

Both *Triticum araraticum* and *Triticum timopheevii* have non-free-threshing hulled ears.

Ecologically, *Triticum timopheevii* is adapted to cool moist alpine climates, being undemanding for warmth and resistant to excessive humidity (Dekapreleovich, 1954). The grain has high bread-making quality (Zhukovsky, 1971). Even in the northern region of Russia (district Leningrad) the protein content has been found to be 19.4% (Tavrin, 1963).

T. militinae was found among the plants of a *Triticum timopheevii* collection plot by Zhukovsky in 1950 (Jakubziner, 1969; Zhukovsky and Migushova, 1969). The plants are 100–120 cm in height, a little shorter than *T. timopheevii* plants. Morphologically, the vegetative organs of *T. militinae* resemble those of *T. timopheevii*, with the exception of the awn-pointed slightly carinate empty glumes which are considered to be similar to those of *T. persicum* var. *fuliginosum* (Zhukovsky and Migushova, 1969). The ears are dark, and are shorter (3.5–4.5 cm), wider and even more compact (50–70 spikelets/10 cm of spike rachis; Dorofeev, 1976) than those of *T. timopheevii*, differing from the latter in having naked grains and black awns (3–5 cm). *T. militinae* is the only free-threshing tetraploid species in the *Boeoticum* subgenus.

T. militinae is considered to be a spontaneous mutant of *T. timopheevii* (Dorofeev, 1976), but it has also been supposed to originate from an introgressive hybridization between *T. timopheevii* and *T. persicum* (Navruzbekov, 1981).

All three timopheevii wheats cause cytoplasmic male sterility when crossed with *T. aestivum* (Wilson and Ross, 1962; Maan and Lucken, 1968).

Dorofeev (1976) referred to 43 accessions of *T. timopheevii* and one of *T. militinae* in the World Wheat Collection of the Vavilov Institute of Plant Industry, St Petersburg, Russia. At present, the collection includes 23 accessions classified according to their morphological characteristics as *T. timopheevii*, 11 accessions classified as *T. timopheevii* var. *timopheevii*, 2 accessions classified as *T. timopheevii* var. *typicum* and 5 accessions classified as *T. timopheevii* var. *viticulosum*. The latter accessions of *T. timopheevii* have black awns. Three accessions of *T. militinae* are listed (<http://www.dainet.de/genres/vir/index.htm>).

Chromosomal analysis of *T. timopheevii* and *T. militinae*

The A^t and G genomes of timopheevii wheats can clearly be differentiated by the C-banding technique, the A^t genome being only slightly banded by heterochromatin and the G genome, according to Badaeva et al. (1991), containing more heterochromatin than any other wheat or *Aegilops* species. The assignment of individual A^t and G genome chromosomes to homoeologous groups has been based on the similarity of chromosome banding patterns (Badaeva et al., 1986; Gill and Chen, 1987), chromosome pairing in interspecific hybrids with wheat (Gill and Chen, 1987) and compensating ability in spontaneous substitution lines (Badaeva et al., 1991; Gill et al., 1988). Based on a C-banded chromosome analysis of *T. aestivum* × *T. timopheevii* hybrid lines, Badaeva et al. (1991) developed a classification of the A^t and G genome chromosomes in general use that agrees with the standard genetic nomenclature of *T. aestivum* chromosomes.

T. timopheevii is characterized by high karyotype stability with respect to chromosomal aberrations, having significantly lower variability than its wild relative, *Triticum araraticum*. Nine accessions of *T. timopheevii* have been found to be karyotypically identical (Badaeva et al., 1994b). The high karyotypic stability has been considered to be caused by the relatively young age of the domesticated timopheevii wheat.

T. militinae has been found to be similar in C-banding pattern to *T. timopheevii*, the differences in the *T. militinae* karyotype not exceeding the intraspecific variability of *T. timopheevii* (Badaeva et al., 1994b). However, in an earlier study the same authors indicated that the 1A chromosome of *T. militinae* was slightly longer compared to the same chromosome of *T. timopheevii*, and therefore had a more submetacentral character (Badaeva et al., 1988).

The A and A^t genomes of tetraploid wheats (emmer and timopheevii wheats, respectively) presumably originated from *T. monococcum* L. ssp. *urartu* (Dvorák et al., 1988; 1993). The B and G genomes both supposedly originate from *Ae. speltoides*, having nevertheless a diphyletic origin from different accessions of the latter (Tsunewaki and Ogihara, 1983; Tsunewaki, 1996; Brown-Guedira et al., 1996; Rodriguez et al., 2000; Khlestkina and Salina, 2001; Huang et al., 2002).

A species-specific intergenomic translocation, 6A¹-1G-4G, has been found in *Triticum araraticum* and *Triticum timopheevii*. It was considered that in the raw original amphiploid of *Ae. speltoides* and *T. monococcum*, this translocation arose in order to overcome nucleo-cytoplasmic incompatibility and to restore fertility (Jiang and Gill, 1994).

Studies of pairing at meiosis in the F₁ of *T. aestivum*/*T. timopheevii* and *T. turgidum*/*T. timopheevii* pentaploid interspecific hybrids have shown that A- and A¹-genome homoeologues generally have higher pairing affinity (Feldman, 1966; Gill and Chen 1987; Gill and Sears, 1988). Maestra and Naranjo (1999) analysed chromosome pairing at metaphase-I in the F₁ hybrids from different cross combinations of *T. turgidum*, *T. aestivum* and *T. timopheevii* and also found that, in general, the homologous pairing between the A-genome chromosomes was similar in the three hybrid types AA¹BG, AA¹BGD and AABBD. Associations of B-G chromosomes were found to be less frequent than those of B-B chromosomes.

The pattern and frequency of individual chromosome substitutions in interspecific hybrids is closely related to the respective homoeology of the chromosomes involved in the substitution, that determines their compensation ability. Badaeva et al. (1991; 2000) analysed the general features of chromosome substitutions in *T. aestivum* × *T. timopheevii* hybrids obtained by different cross combinations and found that as a general rule A¹ genome chromosomes substituted for their A-genome counterparts, while G-genome chromosomes substituted for their B-genome homoeologues, the latter substitutions being more frequent.

The pattern and frequency of chromosomal substitutions is determined by the genotype of the parental common wheat cultivar (Shkutina et al., 1988; Badaeva et al., 1991). Most frequently, substitutions involving 2B/2G, 6B/6G and 2A/2A¹ have been detected in *T. aestivum* × *T. timopheevii* hybrids with different wheat backgrounds (Badaeva et al., 1991; 1995b; 2000).

According to the C-banding data, substitutions of the whole chromosome seem to be far more frequent than intergenomic translocations (Badaeva et al., 1991; 2000). However, detailed molecular mapping data may not always agree with C-banding data, as was found when analysing chromosome 6B of the *T. timopheevii*-derived wheat line 146-155-T, carrying the powdery mildew resistance gene *Pm27*. According to C-banding analysis, the whole 6B chromosome was substituted by the *T. timopheevii* chromosome 6G in this line, yet more detailed molecular mapping detected an intercalary translocation of part of the same chromosome (Badaeva et al., 1995b; Järve et al., 2000).

The effect of the parental wheat genotype on the frequency of individual chromosome substitutions may be illustrated by the surprisingly great differences detected in the occurrence and frequency of G/D genome chromosome substitutions in different crosses (Badaeva et al., 1991; 2000). Each of the three crosses of a mutant line of cv. Mironovskaya with *T. timopheevii*, as well as two of the four crosses with *T. militinae* analysed in these studies resulted in at least one G/D substitution. A high frequency of G/D substitutions was also detected in *T. timopheevii*-derived introgressive lines of cv. Novosibirskaya 67 (12 lines analysed, four G/1D substitutions and three G/2D substitutions detected; Badaeva et al., 1991). On the other hand, in the

background of the genotype of cv. Pyrotrix 28 (17 introgressive lines analysed) no G/D substitutions were detected and in *T. timopheevii*-derived introgressive lines of cv. Saratovskaya 29 (13 lines analysed), only one substitution of the G/7D chromosome was detected (Badaeva et al., 1991; 1995b).

Data on the karyotypical analysis of chromosome substitutions in *T. aestivum* × *T. militinae* hybrids are limited to the analysis of the above mentioned four *T. militinae*-derived introgressive lines of a mutant line of cv. Mironovskaya (Badaeva et al., 2000). The pattern of chromosome substitutions seems to be similar to that of *T. timopheevii* crosses. The most frequent substitutions in *T. timopheevii*-derived introgressive lines, 2G/2B, 6G/6B, 1A¹/1A and 7A¹/7A, were detected in two hybrid lines, while 2A¹/2A was found in one line. Two of the lines both had two G/D chromosome substitutions: 1G/1D + 3G/3D and 2G/2D + 5G/5D.

As *T. militinae* is considered to be a spontaneous mutant of *T. timopheevii* and is found to be karyotypically identical to the latter (Badaeva et al., 1994b), the chromosome behaviour of both forms of the species should be similar. However, in earlier experiments, where *T. aestivum* (cv. Chinese Spring (CS) and cv. Saratovskaya 29) was crossed with *T. timopheevii* and *T. militinae* as the female parent, it was found that in F₁ pentaploid hybrids (AA¹BGD, 2n=35) the average number of bivalents at MI of meiosis was reduced by one in crosses with *T. militinae*, compared to crosses with *T. timopheevii* (Table 1). Correspondingly, a higher number of univalents was detected in the pollen mother cells (PMCs) of hybrids with *T. militinae*. The results indicate differences between the *T. timopheevii* and *T. militinae* genomes, resulting in the pairing incompatibility of one *T. militinae* chromosome, compared to the affinity of the *T. timopheevii*/*T. aestivum* chromosome sets. This conclusion was confirmed by the analysis of meiosis in *T. timopheevii* × *T. militinae* crosses, where the average number of bivalents (13.1±0.06) was found to be lower than in *T. timopheevii* or *T. militinae* (both 13.9±0.01, Table 1). In 37% of the meiocytes multivalents represented by closed ring quadrivalents were detected in *T. timopheevii* × *T. militinae* hybrids, indicating reciprocal chromosomal translocations between the *T. timopheevii* and *T. militinae* genomes (Shnaider (Enno), 1986; 1988).

It has been assumed that a gene on the *T. timopheevii* 5G chromosome, homoeologous to the pairing suppressor *Ph1* on the long arm of chromosome 5B in *T. aestivum*, controls the diploid-like chromosome pairing (Feldman, 1966; Martinez et al., 1996). In crosses between CS mutant *ph1b* and both *T. timopheevii* and *T. militinae*, the average number of bivalents was found to be higher than in crosses between CS *Ph1* and the same *timopheevii* wheats, while no significant differences were detected between the *T. timopheevii* and *T. militinae* crosses (Shnaider (Enno), 1989), showing that the homoeologous pairing suppressor gene on chromosome 5G of *T. timopheevii* (and presumably on that of *T. militinae*) is not fully capable of replacing *Ph1*. A slightly higher level of bivalents at MI of meiosis was also found in the CS-nulli-5B-tetra-5A × *T. militinae* cross (Table 1). Ozkan and Feldman (2001) classified the *T. timopheevii* allele of *Ph1* as allowing a high to intermediate level of homoeologous chromosome pairing.

Table 1
Chromosome pairing at metaphase-I in the interspecific F₁ hybrids (Schneider (Enno), 1989)

Genotype	No. of PMCs	Average number per cell			
		bivalents	univalents	multivalents	chiasmata
Saratovskaya 29× <i>T. timopheevii</i>	391	7.9±0.1	16.2±0.20	0.85±0.03	13.7±0.10
Saratovskaya 29× <i>T. militinae</i>	347	6.9±0.1	18.5±0.20	0.78±0.04	12.3±0.10
<i>T. timopheevii</i>	292	13.9±0.01	0.08±0.01	0	26.0±0.05
<i>T. militinae</i>	348	13.9±0.01	0.09±0.02	0	27.1±0.05
Saratovskaya 29	455	20.9±0.01	0.10±0.03	0	41.4±0.08
<i>T. timopheevii</i> × <i>T. militinae</i>	426	13.1±0.06	0.15±0.03	0.37±0.03	27.2±0.06
Chinese Spring× <i>T. timopheevii</i>	130	7.2±0.1	18.30±0.30	0.63±0.07	12.0±0.20
Chinese Spring× <i>T. militinae</i>	32	6.4±0.3	21.50±0.50	0.25±0.07	9.5±0.4
Chinese Spring <i>ph1</i> × <i>T. timopheevii</i>	243	8.3±0.1	14.70±0.30	1.1±0.05	15.4±0.2
Chinese Spring <i>ph1</i> × <i>T. militinae</i>	313	8.7±0.1	14.70±0.20	0.85±0.04	13.2±0.2
Chinese Spring nulli-5B-tetra-5A× <i>T. militinae</i>	184	8.4±0.1	16.00±0.20	0.68±0.05	13.3±0.2

Crossability of *T. aestivum* with *T. timopheevii* and *T. militinae*

The recipient wheat cultivar is generally used as the female parent in wide hybridization programmes. In resistance breeding, *T. aestivum* × *T. timopheevii* and *T. aestivum* × *T. militinae* crosses are used.

The hybridization methods are similar to those for other interspecific wheat crosses. The anthers are removed a few days before dehiscence and the pollen of the male parent is applied while the stigma is receptive. The hybrid grains often shrivel and die if left on the wheat ear. Embryos can be rescued by culturing *in vitro*. Different methods of overcoming pre- and postfertilization barriers may be used (reviewed by Sharma, 1995).

Due to genetic incompatibility and differences in genomic structures, the yield of hybrid grains in crosses of *T. aestivum* with *T. timopheevii* and *T. militinae* is extremely low (Shands, 1941; Allard and Shands, 1954; Skurygina, 1958; Leontjev, 1980; Kobylanskyi and Fadeeva, 1986). For example, in efforts to transfer disease resistance from *T. timopheevii* to a cultivated wheat strain, Chopde and Deodikar (1964) pollinated over 2000 emasculated florets with the pollen of *T. timopheevii*, with a success rate of only about 5%. The seeds obtained were shrivelled and underdeveloped, with a very poor germination rate. In crosses between seven different Georgian common wheat cultivars, as female parents, and *T. timopheevii* not a single hybrid seed was obtained. The failure was thought to have been caused by hybrid lethality conditioned by the interaction of the hybrid necrosis genes *Ne1* and *Ne2* (Naskidashvili, 1984).

In earlier experiments (1979–1981), the common wheat cultivar Saratovskaya 29 was crossed as the female parent with *T. timopheevii* or *T. militinae*. In the crosses with *T. timopheevii*, 5.4% of the 5704 florets pollinated set seed. The percentage of seed set was even lower when *T. militinae* was used

as the male parent. Out of a total of 1030 florets pollinated with *T. militinae*, only 1.2% set seed (Peusha and Shnaider, 1983). However, from 1993 to 1995, 14 different Finnish cultivars of common wheat were crossed as female parents with both *T. timopheevii* and *T. militinae*, and no difference in seed setting was found. Summing up all the crosses with *T. timopheevii*, 5.6% of 4882 pollinated florets set seed. In crosses with *T. militinae* 5.4% of 4511 pollinated florets gave seeds (Peusha, unpublished data). In the present experiments, no significant differences were detected in the crossability of different common wheat cultivars, though a slight variability in the results was found from year to year.

As the female parent, cv. Saratovskaya 29 has also been crossed with the F_1 hybrids *T. timopheevii* \times *T. militinae* and *T. militinae* \times *T. timopheevii*. The crossability with *T. aestivum* depended on which of the parents was used as female in the hybrid cross. The F_1 hybrids *T. timopheevii* \times *T. militinae* had slightly better crossability with *T. aestivum* than either of the hybrid parents, *T. timopheevii* and *T. militinae*, alone: the pollination of 576 florets resulted in 50 seeds (8.6%). In the second cross 198 pollinated florets gave only one seed (Peusha and Shnaider, 1983).

The F_1 hybrids between common wheat and *T. timopheevii* or *T. militinae* are self-sterile and can only be maintained by backcrossing them as females with *T. aestivum*. Self-fertility is usually fully restored after 2–3 backcrosses.

Resistance to fungal diseases

Tetraploid wheats of the timopheevii group are considered to be a useful reservoir of disease resistance genes, which can be transferred into common wheat cultivars *via* sexual hybridization.

Triticum araraticum

According to Dorofeev (1976), the wild form of timopheevii wheat is resistant to powdery mildew and loose smut but has no resistance to leaf (brown) rust (caused by *Puccinia recondita* Rob. ex Desm.), yellow (stripe) rust (caused by *Puccinia striiformis* Westend.) or stem rust (caused by *Puccinia graminis* Pers.). Brown-Guedira et al. (1996) evaluated a collection of 301 wild timopheevii wheat accessions from Armenia, Azerbaijan, Iraq and Turkey for resistance to 6 foliar diseases and 2 arthropod pests. All the tested accessions were resistant to Septoria blotch (caused by *Septoria tritici* Roberge in Desmasz.) and a very high percentage of them were resistant to tan spot (caused by *Pyrenophora tritici-repentis* (Died.) Drechs.). Resistance to leaf rust was more frequent in the collection than resistance to stripe rust, stem rust, or powdery mildew. Only about 10% of the tested accessions were classified as having intermediate to high levels of resistance to at least five pests each.

Sr40

The stem rust resistance gene *Sr40* was transferred to the short arm of common wheat chromosome 2B (Dyck, 1992) from *T. araraticum* chromosome 2G (Pridham, 1939; Allard and Shands, 1954; Atkins, 1967; McIntosh and Gyrfas, 1971; McIntosh et al., 1998; <http://wheat.pw.usda.gov/ggpages/wgc.html>). The short arm of the translocation chromosome has a prominent telomeric C-band derived from chromosome 2G, whereas the C-banding pattern of the long arm is similar to the long arm of chromosome 2B. The exact translocation breakpoint has not been determined. *Sr40* has not been used in wheat improvement (McIntosh et al., 1995).

Triticum timopheevii

The domesticated species *Triticum timopheevii* is considered to have complex resistance to powdery mildew, stem, leaf and yellow rusts, dwarf smut (*Tilletia foetida* (Wallr.) Liro) and loose smut (reviewed by Dorofeev, 1976).

Several successful transfers of resistance gene(s) from *Triticum timopheevii* to bread wheat have been reported (Pridham, 1939; Shands, 1941; Allard, 1949; Watson and Luig, 1958; Atkins, 1967; Peusha et al., 1995, 1996). The inheritance of resistance to stem rust in adult plants (Allard and Shands, 1954) and resistance to physiological races of leaf rust and its inheritance have been described in selections derived from crosses of common wheat and *T. timopheevii* (Maan and McCracken, 1969).

Sr36, Sr37

McIntosh and Gyrfas (1971) identified three genes ($SrTt_1 = Sr36$, $SrTt_2 = Sr37$ and an undesignated third) in hexaploid derivatives obtained from *Triticum timopheevii* crosses. The resistance gene *Sr36* was transferred to the short arm of common wheat chromosome 2B (Allard and Shands, 1954; Nyquist, 1957, 1962) from *Triticum timopheevii* chromosome 2G, while the resistance gene *Sr37* was transferred to the short arm of wheat chromosome 4B from the short arm of chromosome 4G (Gyrfas, 1968; McIntosh and Gyrfas, 1971; McIntosh, 1991). *Sr37* has not contributed to wheat cultivar improvement (McIntosh et al., 1995).

Lr18

A gene for resistance to leaf rust, *Lr18*, has been transferred to the long arm of chromosome 5B of common wheat from *T. timopheevii* (McIntosh, 1983). The translocation involves a telomeric N-band of the long arm of chromosome 5G or 1G (Yamamori, 1994); the translocation chromosome consists of the short arm of chromosome 5B, part of the long arm of chromosome 5B, and a terminal segment derived from chromosome 5G (Friebe et al., 1996). *Lr18* has not been used intensively in common wheat breeding, but only in a few cultivars such as Timvera, South Africa 43 and Red Egyptian (McIntosh et al., 1995).

Lr10

According to Badaeva et al. (2000) the resistance gene *Lr10* also originates from *T. timopheevii*. The reaction to inoculation of *T. timopheevii*-derived wheat lines with differentiating isolates of rust pathogen corresponded to that of *Lr10*.

Pm6

The powdery mildew resistance gene *Pm6* has also been transferred to common wheat from cultivated *T. timopheevii* (Allard and Shands, 1954; Dyck and Samborski, 1968; Jorgensen and Jensen, 1973, Skurygina, 1984). The resistance gene *Pm6* conditions moderate susceptibility at the seedling stage, and a resistance reaction at the third leaf and later stages (Allard and Shands, 1954; Nyquist, 1963; Briggles, 1966). *Pm6* is linked with the stem rust resistance gene *Sr36* (McIntosh and Gyarfas, 1971; McIntosh and Luig, 1973; Jorgensen and Jensen, 1973; McIntosh, 1991). Genetic analysis showed that *Pm6* is located on the long arm of common wheat chromosome 2B, whereas *Sr36* has been mapped on the short arm. The *Sr36/Pm6* transfer has been used in germplasms in North America (cv. Arthur and its derivatives Hand, Kenosha, Roughrider, Venum, Wisconsin Supremo), Australia (cvs. Timvera, Mendos, Timgalen, Cook, Songlen), South Africa (cvs. Dipka, Flamink, Gouritz) Kenya and Ethiopia (McIntosh et al., 1995).

Pm6 was found to be closely linked with the loci *Xbcd135* (1.5 ± 1.4 cM), *Xbcd307* (4.7 ± 2.5 cM) and *Xbcd266* (4.5 ± 2.4 cM) (Liu et al., 1998) and linked to the marker OPV20-2000 at a genetic distance of 3.0 ± 2.2 cM (Wang et al., 2000). Tao et al. (2000) have mapped *Pm6* to the interval *Xbcd135*-2B – *Xpsr934*-2B. These authors found that cv. Timgalen and the common wheat line CI12632/Cc (where *Pm6* was first reported) lacked the critical *T. timopheevii* markers.

Pm2

In addition to *Pm6*, another powdery mildew resistance gene, *Pm2*, was identified in five breeding lines developed from wide crosses with *T. timopheevii* in Russia using powdery mildew tester isolates (Peusha et al., 1995). The authors concluded that both *Pm6* and *Pm2*, detected in these lines, had originated from *T. timopheevii*.

Pm2 is carried by numerous genotypes (Briggles, 1966) and is generally considered to originate from *T. tauschii* (Lutz et al., 1994, 1995). The gene has been located on the short arm of chromosome 5D (Meyer, 1977; McIntosh and Baker, 1970) and is linked with the *Xbcd1871* locus (Ma et al., 1994). The genetic distance of *Pm2* to RAPD marker OP104(1700) is 12.2 ± 3.3 cM (Liu et al., 2000).

Considering the C-banding data, indicating an unexpectedly high frequency of G/D genome substitutions in *T. timopheevii*-derived hybrid wheat, a further analysis of the origin of this resistance gene seems to be necessary.

Pm27

Several sets of introgressive wheat lines showing improved resistance to powdery mildew and leaf rust in the seedling stage have been developed from *T. timopheevii* and *T. militinae* in Harku (Peusha et al., 1995, 1996). The response of these lines to inoculation with 11 differentiating powdery mildew isolates differed from that caused by 16 known major genes for resistance to powdery mildew (Peusha et al., 1995).

It was shown by the C-banding technique that these lines possessed the genetic material of *T. timopheevii* and it was suggested that the resistance to pathogens in these lines was determined by chromosome substitution 6G/6B (Badaeva et al., 1995b). According to the results of monosomic analysis, powdery mildew resistance in the hybrid line 146-155-T is conferred by a single dominant gene located on chromosome 6B (Enno et al., 1998). Electrophoretic analysis of the gliadin fractions in the hybrid line 146-155-T and its parents, described in the same study, also indicated an interspecific translocation (substitution) in chromosome 6B.

Using RFLP and microsatellite analysis, a *Triticum timopheevii* translocation was detected in chromosome 6B of the hybrid line 146-155-T and its breakpoints were located (Järve et al., 2000). The results of the hybridization of the rDNA intergenic spacer fragment showed that the region flanking the *NorB2* locus in the introgression line originated from chromosome 6G of *T. timopheevii* and had been transferred into 146-155-T by homoeologous recombination. Linkage was detected between the microsatellite locus *Xpsp3131*, located on the introgressed segment, and *Pm27*.

Triticum militinae Zhuk. et Migush.

Immunity to powdery mildew, leaf and yellow rusts, high resistance to stem rust, loose and dwarf smuts have been reported as the useful traits of *T. militinae* (Dorofeev et al., 1979).

In three different climatic regions, *T. militinae* was found to be unsusceptible to fungal diseases; only traces of stem rust were detected (Dekapreleevich, 1954; Jakubziner, 1969; Migushova, 1975).

T. militinae has rarely been used to improve disease resistance in common wheat. In Harku, common wheat cv. Saratovskaya 29 was crossed with the hybrids *T. timopheevii* × *T. militinae* and *T. militinae* × *T. timopheevii* (Peusha and Shnaider, 1983). The latter cross gave a set of lines of common wheat phenotype with improved resistance to powdery mildew (Peusha et al., 1995). Five of these lines were included in the World Wheat Collection of the Vavilov Institute of Plant Industry, St. Petersburg, as accessions I-0121782–I-0121786. According to C-banding, these lines have various translocations/substitutions derived from the *T. timopheevii*/*T. militinae* genomes. Due to the similar C-banding patterns of *T. timopheevii* and *T. militinae* chromosomes, the exact origin of these translocations/substitutions from the *T. timopheevii* or *T. militinae* genome could not be established (Badaeva et al., 1995b).

Several populations selected from the progenies of crosses between Finnish wheat cultivars Tähti, Fagott, Laari and Troll and *T. militinae* show improved resistance to powdery mildew and leaf rust both at the seedling stage and as adult plants. Tests of allelism and molecular mapping data indicate at least two unidentified major genes for powdery mildew resistance (unpublished data).

Conclusions

Although the karyotypes of *T. timopheevii* and *T. militinae* are almost identical, their chromosome behaviour at meiosis in the F_1 hybrids shows pairing incompatibility for one of the *T. militinae* chromosomes, compared to the *T. timopheevii* chromosome set. If the morphological differences between the two timopheevii wheats are also taken into account, it may be assumed that *T. militinae* has arisen as a result of an introgressive hybridization.

Both *T. timopheevii* and *T. militinae* can still be considered to be valuable sources for the further improvement of disease resistance in common wheat. Although six to eight genes of *T. timopheevii* origin for resistance to different fungal diseases have already been introgressed into common wheat, recent unpublished data indicate at least two more major powdery mildew resistance genes in *T. timopheevii*-derived lines of Finnish common wheat cultivars. Special attention should also be paid to the adult plant resistance to powdery mildew and leaf rust in the above-mentioned derivatives of *T. timopheevii*.

Although *T. militinae* is completely resistant to fungal diseases, as is *T. timopheevii*, it has rarely been used in resistance breeding. It has yet to be discovered how differences in the genome structure of the two timopheevii wheats affect the immunological behaviour of *T. militinae* and its crossability with *T. aestivum*.

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Book review

G. G. KHACHATOURIAN, A. MCHUGHEN, R. SCORZA, WAI-KIT NIP AND Y. H. HUI (Eds.): *Transgenic Plants and Crops*. Marcel Dekker, Inc., New York, Basel, 2002. 867 pp. ISBN 0-8247-0545-9

Enormous progress has been made in transgenic plant production since work began in this field of science. However, right from the beginning, the application of this method and the cultivation of transgenic plants have divided both public and scientific opinion. Some scientists see in this method the solution to the food shortage problems expected to arise due to population growth, while others feel that the environmental risks thought to be involved with transgenes will endanger humanity. The debate is still raging on a wide scale and clear thinking is hindered by the fact that most of those taking part in the debate have no real knowledge on the processes and techniques involved. This book therefore undertakes an extremely important task when it presents the whole spectrum of transgenic plant production, divided into four main topics.

In the first section, "Principles and Applications", the book gives a comprehensive view of the subject. After a brief outline of the development of agriculture it provides a detailed survey of related fields of science which are of key importance for transgenic plant production because they determine the fate of the transgenes. These include a discussion of the dynamic (not static) nature of the genome, the tissue culture methods essential for transformation, the ontogenetic development of the plant, and the technologies employed for the identification, isolation and multiplication of the genes. Many parts of this section provide detailed information on gene transfer techniques and the use to which each

is put. After a discussion of basic principles and general methodological matters, the authors present examples of agronomic traits which have already been perceptibly improved using transformation techniques or which are likely to be improved in the near future through the production of transgenic varieties. The final parts of this introductory chapter give a review of the social and environmental problems related to genetic transformation, including a discussion of public opinion on transgenic plants, the patenting of living organisms, possibilities of industrial application and the expected political and environmental effects and consequences.

The three chapters following this general introduction discuss the results achieved in various groups of crops and the difficulties still encountered in research and development. The first of these chapters (Chapter 2, Fruits) deals with the transformation of fruit species, covering both woody and herbaceous species, including those important for human nutrition and for the economy, such as bananas, apples and grapes. Chapter 3 (Vegetables) gives a similar discussion of vegetable species, followed in Chapter 4 (Grains and Other Seeds) by the genetic transformation of field crops grown for their seed. All these chapters provide up-to-date information on the present status of research into the transformation of the given plant and on the problems still to be solved.

The book will be of special interest to advanced students, teachers and scientists in the field of genetic transformation, plant breeding, genetics, molecular biology, plant tissue culture, and plant biotechnology in general.

G. KOVÁCS

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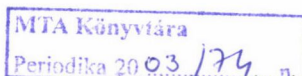
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